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# OBITUARY NOTE

## DR. G. D. BHALERAO, D.Sc. (Lond. et Alld.)

THE sudden death of Dr. G. D. Bhalerao, due to heart failure, on the 6th of December 1948, has deprived India of one of its enthusiastic investigators in the field of helminthological research. He was only 51 at the time of his death.

Dr. Bhalerao was born on the 10th of December 1897 at Wardha and received his early school education in his home town. He, then, migrated to Hislop College, Nagpur, and obtained the B.Sc. degree of the University of Allahabad in 1920. There were no arrangements for higher studies at Nagpur in Zoology in those days. He, therefore, went to join the Muir Central College, Allahabad, to work under Dr. W. N. F. Woodland and took the M.Sc. degree in Zoology. He was a conscientious devotee to the cause of scientific research and in order to widen his outlook he went abroad in 1935. While in England, he joined the School of Hygiene and Tropical Medicine, London, and worked under the distinguished guidance of the leading Helminthologist, Professor R. T. Leiper, F.R.S., now Director, Commonwealth Bureau of Agricultural Parasitology, at St. Albans and obtained the PH.D. and later the D.Sc. from the University of London. He also obtained a D.Sc. degree of the University of Allahabad.

Dr. Bhalerao was interested in teaching and soon after his M.Sc. degree he was selected for an appointment as a lecturer in Biology at Hislop College, Nagpur. Subsequently he served successively on the staff of the University College, Rangoon (Burma) and College of Science, Nagpur. It was at Rangoon that he came in contact with Professor F. J. Meggitt who moulded his career and created his interest for research in Helminthology. He published his first few papers on the Trematode Parasites of Vertebrates under the guidance of his chief. His enthusiasm for helminthological research was likely to grow under suitable conditions and he little knew that it would result in a change of his profession. In 1930, he finally gave up teaching Zoology and accepted the post of a Helminthologist at the Indian Veterinary Research Institute, Mukteswar, Kumaon Hills, U.P., which institution he served till his death. Here he had ample opportunities to carry on researches in his favourite subject. There was a huge collection of helminth materials received from all over the country and library and laboratory facilities which could not be had at any place, not only in the country but perhaps in the east. It was best equipped for research. Under such favourable conditions he devoted himself wholeheartedly to research in helmintholcgy. As a result of his investigations on the collection at Mukteswar, he published several papers describing new forms and gave a revised diagnosis of

those forms that were already known. Finally, in 1935, he published a valuable Monograph on the " Helminth Parasites of Domesticated Animals in India," which work he was, at the time of his death, revising for a second edition under the directions of the Indian Council of Agricultural Research. Needless to say that this monograph is widely used by the investigators in the veterinary departments of the country. Although it has its inherent defects in lacking suitable keys, it is also used by the beginners in helminthological studies at the Universities as one of the books of references.

In 1938, Dr. Bhalerao was entrusted with the arduous task of compiling the " Fauna of Britsh India Volume on Trematoda," a work for which he had amassed an enormous collection of material and literature. I understand this work, at the time of his death, was in a fairly advanced stage of preparation. He had made voluminous index cards with figures and descriptions of the various genera and species already known and also brief summaries of the new forms which he intended to describe. It will, therefore, be in the fitness of things if the authorities recognise the work of his several years' labour and pass it on to a senior helminthologist in the country so that the wealth of knowledge obtained and discovered by Bhalerao may be suitably collated.

Ever since his appointment in 1930, Dr. Bhalerao had been connected with the Indian Institute of Veterinary Research, where he occupied severally the positions of a Helminthologist, an Officer-in-charge of the Veterinary Zoology and at the time of the partition of India, as Officiating Director of the said Institute. He carried out all his duties on these posts with conspicuous success and always earned approbation from his superiors.

Dr. Bhalerao was a member of several scientific societies and Government committees. He presided over the Zoology section of the Indian Science Congress in 1947 and subsequently was the President of the Medical and Veterinary Section of the same body in 1948. He thus attained a fairly eminent position in the scientific field in the country.

Dr. Bhalerao had a thorough knowledge of the systematic Helminthology. He was particularly interested in the field of Veterinary Helminthology which he expressed in his numerous original researches published in various journals, foreign and Indian (A list of his published researches is appended). In one of his contributions, Dr. Bhalerao reports the occurrence of *Schistosoma japonicum* from pigs in India. If this is correct there seems to be a danger of the spread of this infection to man, as it is transmitted to man through the intermediary snail host in China and Japan and may possibly infect man here also. A note of warning must, therefore, be given to those

interested in Public Health in the country to guard against this serious menace to human population as well. In his Presidential Address to the Section of Zoology in 1947, he ably summarised the "Position of Applied Helminthology in India" and brought forward the points that need further elucidation, thereby stressing the need for intensive research in the country. In the meetings of the same section he arranged a symposium on the study of Parasitology in India and thus brought forth the necessity for establishing a research centre in Parasitology on a national basis.

Under the inspiring guidance of Professor R. T. Leiper in England, Bhalerao's love for research in Helminthology increased and he devoted more and more time to the study of control measures. For this purpose he also paid a visit to America to study the latest American methods of control against helminthic infections of the domesticated animals, but unfortunately his results on the application of such methods under Indian conditions could not be made available to us.

During the multifarious activities as a Helminthologist, Dr. Bhalerao was associated with me in several committees, and in discussions on important problems connected with the organisation of helminthological research under the Indian Council of Agricultural Research of the Government of India and helminth control it was always a pleasure to note the thoroughness with which he discussed them. Although a bit excitable in temperament, he was always amenable to reason. A little before his death, I had an opportunity of discussing with him the desirability of establishing a Helminthological Society of India and starting a Journal of Helminthology and we both came to the conclusion that for promoting adequate research in the subject in the country both these were extremely necessary. They both started work during his life-time but he is no longer to nourish this infant body.

In the present transitional stage of our country when India's Sciences are being re-organised under National conditions, his presence will be greatly missed. Personally, I was looking forward to his advice in the organisation of the Helminthological Society of India, and the *Indian Journal of Helminthology*. A recent move for the establishment of a National Research Laboratory for Parasitology (in which he was deeply interested during the latter part of his life) has been denied his support by his sudden death.

It is not easy to assess the extent of the loss that Indian Helminthology has sustained by his sudden death, but he has left behind a wealth of knowledge which is likely to keep his memory alive for a long time to come.

*Department of Zoology,*
*Lucknow University, Lucknow.* G. S. THAPAR.

## A List of Research Publications of the late Dr. G. D. Bhalerao

1924 "A contribution to the knowledge of the trematode parasites of the food mammals of Rangoon." *Ann. Trop. Med. and Parasit.*, vol. 18.

1926 "The intestinal parasites of the bat, with a list of the trematodes hitherto recorded from Burma." *Jour. Burma Res. Soc.*, vol. 15.

"On the synonymy of the genera *Isoparorchis* and *Leptolecithal*, with a description of the male genitalia of *Isoparorchis trisimilitubis*." *Ann. Mag. Nat. Hist.*, vol. 17.

"The trematodes of *Corvus insulens*, with a description of four new species." *Parasit.*, vol. 18.

"A new species of trematode from *Nycticejus pallidus*, with a key to the species of *Lecithodendrium*." *Ann. Mag. Nat. Hist.*, vol. 18.

"On the trematodes of the digestive tract of a common Indian frog, *Rana tigrina*, with a description of *Centrovitus pentadelphi* n.g., n.sp." *Parasit.*, vol. 18.

1927 "A new species of trematode from *Mabuia dissimilis*." *Ann. Mag. Nat. Hist.*, vol. 3.

1929 "The genus *Paradistomum* in Burmese Reptiles." *Ibid.*, vol. 3.

1931 "Trematode parasites of pigs in Bengal." *Rec. Ind. Mus.*, vol. 33.

"Two new trematodes from Reptiles." *Parasit.*, vol. 23.

"A new species of Acanthocephala from *Ophiocephalus striatus*." *Ann. Mag. Nat.* Hist., series 10, vol. 7.

"Two new parasites from king cobra." *Ibid.*, ser. 10, vol. 8.

1932 "On some nematode parasites of goats and sheep at Mukteswar." *Ind. Jour. Vet. Sci.*, vol. 2.

"On the identity of the Schistosomes found in cases of bovine nasal granuloma and some observations on a few other members of the family Schistosomidæ." *Ibid.*, vol. 2.

"A note on the probability of infection of man and Carnivores by *Isoparorchis hypselobagri*." *Ibid.*, vol. 2.

1933 "On two unrecorded nematodes from the abomassum of cattle in India." *Ibid.*, vol. 3.

"The most practical methods of combating parasitic gastritis and fluke infestation of ruminants under field conditions in India." *Agric. and Livest. in India.*, vol. 3.

"A new species of *Gongylonema* from the domestic fowls." *Ind. J. Vet. Sci.*, vol. 3.

"The trematode parasites of the Indian elephant, *E. indicus*." *Ibid.*, vol. 3.

"Preliminary note on the life-history of the common liverfluke, *Fasciola gigantica* in India." *Ibid.*, vol. 3.

1934 "On the occurrence of *Schistosoma japonica* in India." *Ibid.*, vol. 4.

"On the nematode causing stomach tumours of the *Crocodilus palustris*." *Ibid.*, vol. 4.

1935 "On two monostomes from Avian hosts in India." *Ibid.*, vol. 5.

"Anthelmintics for the worms occurring in the alimentary canal of horse." *Ibid.*, vol. 5.

"Helminth Parasites of Domesticated Animals in India." Monograph No. 6. Indian Council of Agricultural Research.

1936 "Studies on the Helminths of India. Trematoda. I–IV." *Jour. Helm.*, vol. 14.

"On some representatives of the Cestode genus *Avitellina* from India." *Ibid.*, vol. 14.

1937 "On the trematode parasites of a water snake, *Tropidonotus piscator*." *Parasit.*, vol. 18.

"On *Pneumotrema travassosi* n.g., n. sp. and two other trematode parasites from the animals dying in the Zoological Society's gardens during 1936–37." *Proc. Zool. Soc.*, vol. 107.

"Schistosomes and Schistosomiasis in India." Moscow.

"On a remarkable Acanthocephala from a fowl in India." *Proc. Zool. Soc.*, vol. 107.

1938 "On a new trematode *Travassostomum natritis* from the intestine of the Indian snake, *Natrix piscator*." *Liv. Jub. Prof. Travassos* (1938).

1939 "A few unusual Helminths of domestic animals in India." *Ind. J. Vet. Sci.*, vol. 9.

"A brief survey of the development of the knowledge of Trematode fauna of India." *Vol. Jub. Prof. Yoshida.*, vol. 2.

1941 "On the occurrence of the bat fluke, *Prosthodendrium ovimagnosum* in dog." *Rec. Ind. Mus.*, vol. 43.

"*Subulura minetti* n.sp. from an Indian fowl." *Proc. Ind. Acad. Sci.*, vol. 14.

1942 "On some Trichostrongyles of domestic ruminants in India." *Parasit.*, vol. 34.

"On two helminths of domestic ruminants in India." *Ibid.*, vol. 34,

"Some metacercarial forms of Clinostomatidæ from India." *Proc. Ind. Acad. Sci.*, vol. 16.

"On a new species of trematode from a spoonbill in Afghanistan." *Ibid.*, vol. 34.

"On Strigeida from India." *Rec. Ind. Mus.*, vol. 45.

1943 "The cercarial fauna of the irrigated tract of the Nizam's Dominions with suggestions regarding their relationship to the trematode parasites in man and domestic and other animals." *Ind. J. Vet. Sci.*, vol. 13.

"On two trematodes from fishes in India." *Proc. Ind. Acad. Sci.*, vol. 18.

1944 "Some helminth parasites of poultry." *Ibid.*, vol. 20.

"Plant Nematodes, a neglected subject in India." *Curr. Sci.*, vol. 13.

"On the occurrence of *Psilochasmus longicirratus* in *Nyroca ferina* in India." *Proc. Ind. Acad. Sci.*, vol. 20.

1945 "Interesting mode of the life-cycle of the lungworm, *Varestrongylus pneumonicus*." *Curr. Sci.*, vol. 14.

"*Loimos secundus* from the gills of the common Indian shark (*Scoliodon sorrokowah*)." *Proc. Ind. Acad. Sci.*, vol. 22.

1947 "Applied Helminthology, its past and future in India." Presidential Address. *Ind. Sci. Cong.*

1948 "Blood Fluke Problem in India." *Ind. Sci. Cong.*, Patna (Presidential Address).

# SULPHUR REQUIREMENT OF *PESTALOTIA MALORUM* AND *PESTALOTIA PSIDII*

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Received November 14, 1949

(Communicated by Prof. S. Ranjan, F.A.Sc.)

## Introduction

It has been shown by Armstrong (1921), Kossowicz and Loew (1912), Volkonsky (1933, '34) and others that fungi can grow successfully on a large number of sulphur compounds. Mothes (1938) found that sulphur metabolism of *Aspergillus niger* was more or less similar to that of green plants, and it differed from that of Yeast. Steinberg (1941) reported that during the metabolism of *Aspergillus niger* sulphur was reduced to sulphoxylate prior to its conversion to an organic sulphur compound. According to him sulphide and disulphide were not assimilated by it. Leonian and Lilly (1938) working with a number of Saprolegniales observed that magnesium sulphate was a poorer source of sulphur than l-cystine. Volkonsky (1933, '34), Dayal (1942), Bose (1943) and Bhargava (1945) obtained similar results with other fungi also. Fischer* went still further and classified the organisms into two categories on the basis of their sulphur metabolism. Those which obtain their sulphur from sulphate ions were called "Euthiotrophic" and those which were unable to utilise sulphate ions were called "Parathiotrophic".

From the preceding observations it becomes obvious that our knowledge as regards relative importance of different sulphur compounds is very inadequate. Further there is much experimental evidence to show the importance of sulphur in the nutrition of fungi. It was, therefore, thought desirable to study the sulphur requirement of *Pestalotia malorum* and *P. psidii.*

## Material and Methods

The material used consisted of *Pestalotia malorum* isolated from rotten apples and *Pestalotia psidii* isolated from mummified guavas. Single spore cultures of these were prepared by the usual method. The stock cultures were maintained on oat meal agar medium.

* Lwoff, A. (1932), "La nutrition des Protozoaires," *Monogr. Inst. Pasteur*, Paris, Masson editeur.

Richard's solution which had magnesium sulphate as source of sulphur was selected as control. It was worked out that Richard's solution contained 0·665 gm. of elemental sulphur per litre of solution. In order to provide identical quantities of sulphur in each series the amount of elemental sulphur in each sulphur compound was calculated so as to furnish 0·665 gm. of elemental sulphur per litre of solution in every case. A series without any sulphur compound was also included.

Throughout the investigation Pyrex glasswares, double distilled water and as far as possible chemicals of analytical grade or purest available were used. Fifty c.c. of the nutrient solution was placed in 150 c.c. Erlenmeyer flasks and sterilised in an autoclave at 15 lbs. pressure for 15 mts. For each series four replicates were used and inoculations were made by the agar disc method as suggested by Garrett (1936). The cultures were incubated at 20° C. for two weeks. At the end of this period the mycelium and spores of each flask were collected separately on previously dried and weighed Whatman No. 42 filter-paper. The fungus was dried in an oven at 60° C. for 72 hours. Subsequently it was transferred to a desiccator and weighed quickly. This weight was used as a quantitative measure for the growth of the fungus on different solutions.

## Observations

The weight of the fungus obtained with different sulphur compounds is given in Table I, in which different inorganic sulphur compounds are arranged in order of sulphate and its products of reduction.

Table I. *Growth of* Pestalotia *spp. after two weeks incubation at* 20° *C.*

| Sources of sulphur | Formula | Average yield of | |
|---|---|---|---|
| | | *P. malorum* | *P. psidii* |
| | | gm. | gm. |
| INORGANIC | | | |
| Potassium persulphate .. | $K_2S_2O_8$ | 0·1318 | 0·1507 |
| ,, sulphate .. | $K_2SO_4$ | 0·4683 | 0·4066 |
| Magnesium sulphate .. | $MgSO_4$ | 0·5061 | 0·4303 |
| Sodium bisulphite .. | $NaHSO_3$ | No growth | No growth |
| ,, hyposulphite .. | $Na_2S_2O_4 \cdot 2H_2O$ | No growth | No growth |
| ,, metasulphite .. | $Na_2S_2O_5$ | 0·0681 | 0·0600 |
| ,, disulphide .. | $Na_2S_2$ | 0·0925 | 0·1132 |
| ,, sulphide .. | $Na_2S \cdot 9H_2O$ | 0·2809 | 0·2405 |
| ,, thiosulphate .. | $Na_2S_2O_3 \cdot 5H_2O$ | 0·0907 | No growth |
| ,, dithionate .. | $Na_2S_2O_6$ | 0·1885 | 0·2167 |
| ORGANIC | | | |
| Thiourea .. | $CS \cdot NH_2 \cdot NH_2$ | 0·2503 | 0·2019 |
| Cystein .. | $S \cdot CH_2 \cdot CH(NH_2)COOH$ | 0·5611 | 0·5345 |
| Thioglycollic acid .. | $HS \cdot CH_2 \cdot COOH$ | No growth | No growth |
| No sulphur .. | | No growth | No growth |

It is observed from the above table that some source of suitable sulphur is necessary for the organisms. There is no growth in complete absence of any sulphur or when sodium bisulphite or sodium hyposulphite or thioglycollic acid is used for supplying the sulphur needs of the fungi. It is also seen that different chemical substances both organic and inorganic show marked differences in the weight of the organisms even when the quantity of sulphur supplied by them is similar. In general, *P. psidii* gives a lower dry weight than *P. malorum* on the same medium but it is noticed that on potassium persulphate, sodium disulphide and sodium dithionate the growth of *P. psidii* is slightly better than that of *P. malorum*. The best growth was observed on cystein (an organic sulphur compound) and next best on magnesium sulphate (an inorganic sulphur compound). Slight growth of *P. malorum* is possible on sodium thiosulphate, though on this *P. psidii* does not grow at all. Persulphate, metasulphite and disulphide are also only feebly utilised by both the fungi. These results, therefore, show that sulphur requirement of both the species of *Pestalotia* is not similar as they respond differently to different sulphur compounds.

## Discussion

The assimilation of sulphur and sulphur compounds by fungi had been a subject of much investigation for a long time. The importance of sulphur in the nutrition of *P. malorum* and *P. psidii* was exhibited by the fact that there was no growth when sulphur was absent. Naegeli (1880) also observed sulphur and phosphorus to be indispensable for the growth of the moulds on which he was working.

The organisms under investigation exhibited selectivity in the absorption of the different sulphur compounds. In some instances where the source of sulphur was not favourable the development was either poor or totally absent.

Among the inorganic sulphur compounds used maximum yields were obtained with sulphates. Mosher, *et al.* (1936) working with *Trichophyton interdigitale* observed sulphate as one of the most essential ion for its growth. Armstrong (1921) observed that persulphate, sulphite and sulphydryl could be substituted for sulphate in the nutrition of *Aspergillus niger*, *Penicillium glaucum* and *Botrytis cinera*. Volkonsky (1933, '34) made similar observations with certain water moulds. Schade (1940) on the other hand found that *Leptomitus lacteus* and *Apodachlya brachynema* filled their sulphur requirements by reducing sulphates. In these investigations, however, it was observed that sulphate could not be profitably replaced by any other inorganic sulphur compounds. Sulphide, dithionate and persulphate

supported some growth of both the fungi, but on rest of the compounds, the organisms either failed to grow or their growth was extremely poor. Steinberg (1939) studying the nutritional requirement of species of *Saprolegnia, Isoachlya, Achlya, Dictyuchus* and *Aphanomyces* observed sulphate to be the best source of sulphur. He found that thiosulphate, sulphydryl and sulphide could also be utilised to some extent.

As regards the utilisation of the sulphur compounds by *P. malorum* and *P. psidii* it was observed that cystein was an excellent source. Results with cystein were even better than those with sulphates. Volkonsky (1933, '34) observed that certain species of Saprolegniaceæ were unable to use sulphates but thrived well on organic sulphur compounds. The results for organic sulphur were substantiated by Leonian and Lilly (1938) whose experiments evinced that amino acid l-cystine was necessary for the growth of *Saprolegnia mixta, Achlya conspicua, Aphanomyces camptostylus* and *Isoachlya monilifera.* Steinberg's (1941) experiments, however, showed that *Aspergillus niger* utilised both organic and inorganic sulphur compounds. Of the organic sulphur compounds alkyl sulphonates and alkyl sulphinates were readily assimilated but the alkyl mercaptans, sulphides and disulphides were not profitably utilised. In the case of inorganic sulphur compounds the sulphur was first reduced to sulphoxalate and then it was converted to organic sulphur. Such a differential utilisation of sulphur compounds was also noticeable in the present investigation. Among the organic compounds used cystein was utilised most, thiourea supported only moderate growth of the organisms while no growth was observed with thioglycollic acid.

In spite of the fact that in each series the quantity of sulphur, nitrogen, carbon, etc., supplied was the same it was noticed that the organisms did not maintain the same uniformity in growth. In fact, different weights were recorded with different media.

## Summary

*P. malorum* and *P. psidii* isolated from rotten apples and mummified guavas respectively, gave different results with different sulphur compounds. Maximum yield of both was observed on sulphates or cystein. On thiourea, growth was moderate, but sulphide, dithionate and persulphate were only feebly utilised. On other sulphur compounds used in the present investigation, the growth was either extremely poor or totally absent.

## Acknowledgments

The author takes great pleasure in expressing his deep sense of gratitude to Dr. R. N. Tandon of the Botany Department, University of Allahabad,

for suggesting the problem and for his valuable suggestions. To Prof. S. Ranjan, Head of the Botany Department, Uuiversity of Allahabad, the author is much indebted for providing laboratory facilities and displaying personal interest in his work.

## References


Armstrong, G. M. .. "Studies in the physiology of the fungi. XIV. Sulphur nutrition, the use of thiosulphate as influenced by hydrogen-ion concentration," *Ann. Mo. Bot. Gadn.*, 1921, **8**, 237–81.

Bhargava, K. S. .. "Sulphur and Phosphorus requirement of Saprolegniaceæ," *Proc. Nat. Acad. Sci.*, 1945, **21**, 344–49.

Bose, S. K. .. Unpublished thesis, Univ. of Allahabad, 1943.

Dayal, D. .. Unpublished thesis, Univ. of Allahabad, 1942.

Garrett, S. D. .. "Soil conditions and the take all disease of wheat," *Ann. Appl. Biol.*, 1936, **23**, 667–99.

Kossowicz, A. and Loew, W. .. "Über das verhalten von Hefen und Schimmelpilzen zu Natriumthiosulfat," *Ztschr. f. Gärungsphysiol*, 1912, **2**, (**87**), 103.

Leonian, L. H. and Lilly, V. G. .. "Studies on the nutrition of fungi I. Thiamin, its constituents and the source of nitrogen," *Phytopath.*, 1938, **28**, 537–48.

Mosher, W. A., Saunders, D. H., Kingery, L. B. and Williams, R. J. "Nutritional requirements of the pathogenic world, *Trichophyton interdigitale*," *Plant Physiol.*, 1936, **11**, 795–806.

Mothes, K. .. "Über den Schwefelstoffwechsel der pflanzen," *Planta.*, 1938, **29**, 67–109.

Naegeli, C. von .. "Ernährung der niederen Pilze durch Kohlenstoff und Stickstoff-verbindungen, K. b. Akad. wiss. *München Sitzenber*, 1880, **10**, 267–77.

Schade, A. L. .. "The nutrition of Leptomitus," *Amer. J. Bot.*, 1940, **27**, 376–84.

Steinberg, R. A. .. "Growth of fungi in synthetic nutrient solutions," *Bot. Rev.*, 1939, **5**, 327–50.

———— .. "Sulfur and trace element nutrition of *Aspergillus niger*," *J. Agric. Res.*, 1941, **63**, 109–127.

Volkonsky, M. .. "Sur les conditions de culture et le ponvoir de synthèse de *Saprolegnia sp.* Étude qualitative de l'alimentation carbonee, Azotée et sulfurée," *Ann. Inst. Pasteur*, 1933, **50**, 703–30.

———— .. "Sur la nutrition de quelques champignons saprophytes et parasites," *Ann. Inst. Pasteur*, 1934, **52**, 76–101.

# FURTHER OBSERVATIONS ON THE EFFECT OF SOME SUBSTANCES ON THE CONTRACTILE MECHANISM OF UNSTRIATED MUSCLE

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Singh and Singh (1949) have described the action of various substances on the contractile mechanism of unstriated muscle. In the present research, these experiments have been extended and the action of some other substances investigated. The excitatory mechanism of unstriated muscle dies before the contractile system, so that in a dying muscle it is possible to test the action of substances on the latter uncomplicated by effects on the former.

Experimental methods were as described previously; frog's stomach muscle has been used. Some of the experiments were also repeated on mammalian unstriated muscle from dog's stomach. These experiments were performed at 20° C.

## Results

*Effect of sodium hydroxide.*—Sodium hydroxide causes active elongation. Concentrations up to 1 *N* NaOH were tested. Thus in 6 muscles from frog's stomach, the lengths after immersion for 24 hours in isotonic sodium hydroxide solution (0·112 *N* NaOH) and distilled water were 195±34 and 212 ± 30 p.c. of original respectively; after immersion for one hour, these were 201 ± 34 and 126 ± 8 p.c. respectively. The effect of varying the concentration of sodium hydroxide was tested, using 0·025, 0·05, 0·075, 0·1, 0·25, 0·5, and 0·75 *N* NaOH; maximum elongation is produced in 0·75 *N* NaOH. Stronger solutions such as 1 *N* NaOH cause disintegration of the tissue and hence their effects could not be tested after prolonged immersion.

Small concentrations of potassium decrease the effect of sodium hydroxide. Thus in 6 muscles, the lengths after immersion for 24 hours in isotonic sodium hydroxide solution and isotonic sodium hydroxide with the usual amount of potassium chloride as present in the saline, were 173± 27 and 165±23 p.c. of original respectively; after immersion for one hour, these were 172±26 and 175±14 p.c. respectively. Small concentration of calcium increase the effect of sodium hydroxide. Thus in 6 muscles, the

lengths after immersion for 24 hours in isotonic sodium hydroxide solution and isotonic sodium hydroxide with the usual amount of calcium chloride as present in the saline, were $178 \pm 44$ and $181 \pm 20$ p.c. of original respectively; after immersion for one hour these were $183 \pm 33$ and $180 \pm 14$ p.c. respectively. Small concentrations of magnesium decrease the effect of sodium hydroxide. Thus in 6 muscles, the lengths after immersion for 24 hours in isotonic sodium hydroxide solution and isotonic sodium hydyoxide with magnesium chloride ($0{\cdot}02\ M\ MgCl_2$) were $187 \pm 27$ and $180 \pm 25$ p.c. of original respectively. After immersion for one hour these were $195 \pm 23$ and $193 \pm 25$ p.c. respectively.

Stretching antagonises the effect of sodium hydroxide. If the muscle is put under tension, and then immersed in sodium hydroxide, it does not relax, though the addition of hydrochloric acid shows that the muscle is capable of relaxation. These effects resemble those as described previously (Singh and Singh, 1950 *b*). The muscle swells when under tension, so that swelling is not the cause of active elongation.

*Effect of hydrochloric acid.*—An isotonic solution of hydrochloric acid ($0{\cdot}112\ N$ HCl) causes active elongation which is less than that caused by distilled water. Thus in 6 muscles, the lengths after immersion for 24 hours in hydrochloric acid and distilled water were $152 \pm 25$ and $195 \pm 39$ p.c. respectively. After one hour's immersion, these were $133 \pm 8$ and $130 \pm 6$ p.c. respectively. 1 *N* HCl causes contraction. Thus in 6 muscles the lengths after immersion for 24 hours in 1 *N* HCl and distilled water were $86 \pm 6$ and $187 \pm 23$ p.c. respectively; after one hour's immersion these were $86 \pm 3$ and $131 \pm 14$ p.c. respectively. Thus the strong acid solution causes maximum contraction in about an hour. This is because substances that kill the muscle act immediately on the contractile mechanism.

Both the sodium hydroxide ($0{\cdot}112\ N$) and hydrochloric acid (I *N* HCl) kill the muscle, but the muscle contracts and elongates actively, if alternately placed in hydrochloric acid and sodium hydroxide respectively for 24 hours. This can be done for 6 days, after which the experiment has to be stopped owing to disintegration of the tissue (Table I). Thus dead muscle can be made to contract; the dead proteins contract and elongate and the effect of acid and alkali would only be a direct one on the contractile mechanism of unstriated muscle.

If the muscle is immersed in distilled water, replacement in hydrochloric acid after 24 hours, does not cause contraction, showing that the contractile mechanism is permanently damaged by distilled water, but not by weak alkali solution.

TABLE I

*Effect of* 0·112 *N NaOH and* 1 N *HCl alternately on length of frog's stomach muscle. Immersed in each solution for* 24 *hours*

| No. of experiment | Length in p.c. | | | | | |
|---|---|---|---|---|---|---|
| | Initial | in NaOH | in HCl | in NaOH | in HCl | in NaOH |
| 1 | 100 | 170 | 135 | 155 | 130 | 155 |
| 2 | 100 | 230 | 150 | 220 | 175 | 220 |
| 3 | 100 | 144 | 96 | 151 | 104 | 141 |
| 4 | 100 | 226 | 158 | 242 | 179 | 242 |
| 5 | 100 | 209 | 170 | 204 | 152 | 204 |
| 6 | 100 | 190 | 129 | 181 | 133 | 191 |

Small concentrations of potassium decrease the effect of hydrochloric acid (0·112 *M* HCl). Thus in 6 muscles, the lengths after immersion for 24 hours in isotonic hydrochloric acid solution and hydrochloric acid with the usual amount of potassium chloride as present in the saline, were 172 ± 21 and 156 ± 17 p.c. respectively; after one hour's immersion they were 153 ± 15 and 151 ± 25 p.c. respectively. Small concentrations of calcium increase the effect of hydrochloric acid. Thus in 6 muscles, the lengths after immersion for 24 hours in isotonic hydrochloric acid solution and hydrochloric acid with the usual amount of calcium chloride as present in the saline were 153 ± 32 and 158 ± 31 p.c. respectively; after one hour's immersion these were 137 ± 20 and 145 ± 20 p.c. respectively. Small concentrations of magnesium act like calcium; they increase the effect of hydrochloric acid. Thus in 6 muscles: the lengths after immersion for 24 hours in isotonic hydrochloric acid and isotonic hydrochloric acid with magnesium (0·02 *M* $MgCl_2$), were 141 ± 20 and 149 ± 8 p.c. respectively; after one hour's immersion they were 141 ± 20 and 153 ± 20 p.c. respectively.

*Effect of concentrated acids and alkalies.*—Saturated solution of sodium hydroxide causes contraction, whilst concentrated acids such as hydrochloric, sulphuric, nitric, phosphoric, lactic, acetic, propionic and butyric, cause active elongation. In some of these acids, such as sulphuric and nitric, the muscle dissolves; in phosphoric acid it dissolves more slowly.

*Effect of other alkalies.*—Isotonic solution of potassium hydroxide causes active elongation. Thus in 6 muscle the lengths after immersion for 24 hours in 0·112 *M* KOH and distilled water were 176 ± 23 and 197 ± 19 p.c. respectively; after immersion for one hour these were 177 ± 19 and 136 ± 9 p.c. respectively. Thus in one hour, both the sodium and

potassium hydroxides cause more elongation than distilled water. In 24 hours, they contract somewhat.

Isotonic sodium carbonate solution causes active elongation. Thus in 6 muscles the lengths after immersion for 24 hours in 0·075 *M* $Na_2CO_3$ and distilled water were 236 ± 9 and 199 ± 39 p.c. respectively; after immersion for one hour, the lengths were 112 ± 12 and 141 ± 14 p.c. respectively. Thus sodium carbonate causes the maximum elongation of all substances tested so far.

*Effect of other acids.*—The effect of isotonic solutions of following acids was tested:—sulphuric, nitric, phosphoric ($H_3PO_4$), oxalic, lactic, acetic, propionic and butyric. They cause active elongation. Their effect is shown in Table II. They all cause more elongation than distilled water with the exception of nitric acid.

TABLE II

*Effect of isotonic solutions of certain acids on length of frog's stomach muscle*

| No. | Name of Acid | Concentration *M* | No. of Experiments | Length; p.c. | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Initial | In $H_2O$ | | In acid | |
| | | | | | 1 hr. | 24 hrs. | 1 hr. | 24 hrs. |
| 1 | Sulphuric | 0·075 | 6 | 100 | 130 ± 15 | 171 ± 19 | 136 ± 12 | 148 ± 24 |
| 2 | Nitric | 0·112 | 6 | 100 | 150 ± 10 | 210 ± 33 | 139 ± 15 | 139 ± 15 |
| 3 | Phosphoric | 0·056 | 6 | 100 | 131 ± 19 | 203 ± 21 | 168 ± 20 | 184 ± 29 |
| 4 | Oxalic | 0·075 | 6 | 100 | 146 ± 20 | 199 ± 32 | 156 ± 14 | 183 ± 27 |
| 5 | Lactic | 0·112 | 6 | 100 | 123 ± 10 | 173 ± 35 | 158 ± 11 | 215 ± 46 |
| 6 | Acetic | 0·112 | 6 | 100 | 130 ± 25 | 162 ± 27 | 128 ± 25 | 183 ± 16 |
| 7 | Propionic | 0·112 | 6 | 100 | 130 ± 8 | 188 ± 38 | 102 ± 9 | 174 ± 34 |
| 8 | Butyric | 0·112 | 6 | 100 | 126 ± 8 | 172 ± 27 | 122 ± 14 | 184 ± 15 |

*Effect of some sodium salts.*—The effect of following sodium salts was tested:—sulphate, oxalate, citrate, borate, acetate and fluoride. Isotonic solutions were used. Their effect is shown in Table III. They all cause contraction, with exception of fluoride and borate which cause active elongation.

*Effect of heavy metals and protein precipitants.*—The effect of the following substances were tested:—mercuric chloride, zinc sulphate, uranium acetate, sodium tungstate and trichloracetic acid. Their effect is shown in Table IV. They all cause contraction with the exception of trichloracetic acid which causes active elongation.

TABLE III

*Effect of some sodium salts on length of frog's stomach muscle*

| No. | Name of salt | Concentration *M* | No. of Experiments | Length ; p.c. | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Initial | In $H_2O$ | | In salt | |
| | | | | | 1 hr. | 24 hrs. | 1 hr. | 24 hrs |
| 1 | Sodium sulphate | 0·075 | 6 | 100 | 113±16 | 168±16 | 103± 5 | 75± 2 |
| 2 | Sodium flouride | 0·112 | 6 | 100 | 126±14 | 180±37 | 94±10 | 105±19 |
| 3 | Sodium acetate | 0·112 | 6 | 100 | 125±13 | 167±18 | 94± 6 | 93± 5 |
| 4 | Sodium citrate | 0·056 | 6 | 100 | 128± 8 | 184±23 | 108± 8 | 90± 5 |
| 5 | Sodium borate | 0·075 | 6 | 100 | 132±13 | 216±31 | 72± 7 | 113±14 |
| 6 | Sodium oxalate | 0·075 | 6 | 100 | 128±15 | 168±15 | 96± 4 | 93± 7 |

TABLE IV

*Effect of some heavy metals and protein precipitants on length of frog's stomach muscle*

| No. | Name of Substanee | Concentration *M* | No. of Experiments | Length ; p.c. | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Initial | In $H_2O$ | | In substance | |
| | | | | | 1 hr. | 24 hrs. | 1 hr. | 24 hrs. |
| 1 | Mercuric chloride | 0·112 | 6 | 100 | 136±14 | 204±17 | 98±9 | 92±7 |
| 2 | Zinc Sulphate | 0·112 | 6 | 100 | 137±13 | 200±23 | 107±11 | 85±11 |
| 3 | Uranium acetate | 0·112 | 6 | 100 | 143±14 | 185±28 | 96±6 | 94±8 |
| 4 | Sodium tungstate | 0·075 | 6 | 100 | 139±10 | 192±48 | 102±10 | 70±5 |
| 5 | Trichloracetic acid | 0·112 | 6 | 100 | 142±16 | 172±18 | 115±18 | 105±22 |

*Effect of vitamins.*—The effect of thiamine hydrochloride (1 in 100,000), riboflavine (1 in 100,000) and ascorbic acid (1 in 100,000) was tested, the vitamins being dissolved in saline (pH 8, borate buffer). Thiamine causes slight contraction. Thus in 6 muscles, the lengths after immersion for 24 hours in thiamine solution and saline were 87±5 p.c. and 93± 8 p.c. respectively; after one hour's immersion these were 99±7 and 101±6 p.c. respectively. Ascorbic acid has no significant effect. Thus in 6 muscles immersed for 24 hours in ascorbic acid solution and saline, the lengths were 103± 10 and 102±9 p.c. respectively; after one hour's immersion these were 114±13 and 107±12 p.c. respectively. Riboflavine also has no significant action. Thus in 6 muscles immersed in riboflavine solution and saline for

24 hours, the lengths were 96±7 and 94±4 p.c. respectively; after one hour's immersion, these were 94±5 and 95±5 p.c. respectively.

*Effect of glycine.*—Glycine diminishes the effect of distilled water. Thus in 6 muscles immersed for 24 hours in isotonic solution of glycine (0·112 *M*) and distilled water the lengths were 103±12 and 175±15 p.c. respectively; after one hour's immersion, these were 117±13 and 129±13 p.c. respectively. Glycine thus causes contraction.

*Effect of anæsthetics.*—Urethane causes marked elongation just as urea (Singh and Singh, 1949). Thus in 6 muscles, the lengths after immersion for 24 hours in an isotonic solution of urethane (0·224 *M*) and distilled water, were 181±19 and 157±29 p.c. respectively; after immersion for one hour, these were 135±7 and 121±16 p.c. respectively.

A saturated solution of chloralose at 20° C. causes elongation which is less than that of distilled water. Thus in 6 muscles, the lengths after immersion for 24 ours in chloralose solution and distilled water were 120±5 and 171±23 p.c. respectively; after immersion for one hour, these were 156±4 and 127±14 p.c. respectively.

*Effect of sugars.*—Glucose and sucrose cause contraction. Thus in 6 muscles, immersed for 24 hours in isotonic solution of glucose (0·224 *M*) and distilled water, the lengths were 88±9 and 162±30 p.c. respectively; after one hour's immersion these were 112±15 and 144±27 p.c. respectively. In another 6 muscles the lengths after immersion for 24 hours in isotonic solution of sucrose (0·224 *M*) and distilled water were 80±19 and 164±19 p.c. respectively; after immersion for one hour, these were 133±21 and 154±14 respectively.

*Effect of histamine.*—Histamine is a normal constituent of muscle and is known to be liberated as a result of contraction. Compared to saline it causes slight contraction. Thus in 6 muscles immersed for 24 hours in histamine solution (1 in 10,000 in saline) and saline the lengths were 102±18 and 113±8 p.c. respectively; after immersion for one hour these were 101±19 and 106±9 p.c. respectively.

*Effect of adrenaline.*—The effect of adrenaline was tested in saline of pH 6·5 (phosphate buffer). It causes active elongation, when compared to the effect of saline. Thus in 6 muscles immersed in adrenaline (1 in 10,000) and saline for 24 hours, the lengths were 95±4 and 89±11 p.c. respectively; after one hour's immersion, these were 124±9 and 102±5 p.c. respectively. Thus adrenaline causes inhibition in living muscle by active elongation (Singh and Singh, 1950 *a*). 24 hours' immersion shows that adrenaline

counteracts the effect of acid saline, so that it must therefore be causing elongation also by direct action on the contractile mechanism.

*Effect of magnesium.*—As magnesium ions have important effects on actomyosin systems, the effect of small concentrations were tested. Small concentrations of magnesium (0·02 *M* $MgCl_2$) increase the effect of sodium chloride. Thus the length of 6 frog's muscles after immersion for 24 hours in isotonic sodium chloride solution and isotonic sodium chloride with magnesium were 100±12 and 103±7 p.c. of original respectively; after one hour's immersion these were 117±7 and 120±9 p.c. respectively. Small concentrations of magnesium also increase the effect of potassium chloride. Thus the lengths of 6 frog's muscles after immersion for 24 hours in isotonic potassium chloride solution and isotonic potassium chloride with magnesium were 146±10 and 151±10 p.c. of original respectively; after one hour's immersion they were 117±8 and 122±10 p.c. respectively.

*Effect of potassium.*—Effect of varying the concentration of potassium was tested. Thus the length of 6 frog's muscles immersed for 24 hours in isotonic sodium chloride and sodium chloride of which 20 p.c. was replaced with potassium chloride, were 103±3 and 106±4 p.c. respectively; after immersion for one hour these were 103±7 and 109±8 p.c. respectively. Thus potassium causes only elongation.

*Effect on mammalian muscle.*—The action of ions on dog's stomach muscle is more or less similar to that on frog's muscle. Thus after 24 hours' immersion, isotonic potassium chloride (0·154 *M* KCl) causes active elongation. The length of 6 pieces of dog's stomach muscle immersed in potassium chloride were 106, 112, 126, 120, 120, and 100 p.c. of original respectively. Ammonium chloride causes contraction. Thus the lengths of 6 pieces of dog's stomach muscle immersed in ammonium chloride (0·154 *M* $NH_4Cl$) were 98, 90, 80, 80, 90 and 90 p.c. of original respectively. Lithium chloride causes elongation. Thus the lengths of 6 pieces of dog's stomach muscle immersed in lithium chloride (0·154 *M* LiCl) were 125, 115, 100, 112, 118 and 109 p.c. of original respectively. Sodium chloride causes contraction. Thus the lengths of 6 pieces of dog's stomach immersed in isotonic sodium chloride (0·154 *M* NaCl) were 88, 90, 88, 92, 81 and 90 p.c. of original respectively. The monovalent cations therefore cause active elongation of dog's stomach muscle in the order

$$K > Li > NH_4 > Na.$$

The monovalent anions act on the dog's stomach muscle as on the frog's muscle. Thus the lengths of 6 pieces of dog's muscle immersed in isotonic

sodium bromide solution (0·154 *M* NaBr) were 93, 96, 79, 110, 77 and 87 p.c. of original respectively. Sodium bromide thus causes contraction. Lengths of 6 pieces in sodium nitrate were 90, 84, 75, 88, 96 and 80 p.c. of origin respectively. Lengths of 6 pieces in sodium iodide were 87, 92, 104, 82, 77 and 91 p.c. of original respectively. Lengths of 6 pieces in sodium thiocyanate were 105, 116, 100, 100 and 100 p.c. of original respectively. Lengths of 6 pieces in sodium cyanide were 147, 147, 169, 150, 164 and 158 p.c. of original respectively. Thus anions increase the length of dog's stomach muscle in the order

$$Cl = Br = No_3 < I < SCN < CN.$$

Sodium hydroxide causes elongation. Thus the lengths of 6 pieces of dog's stomach muscle immersed in 0·112 *N* NaOH for 24 hours were 141, 123, 123, 150, 140 and 140 p.c. of original respectively. After immersion in 1 *N* HCl, they contracted to 127, 113, 116, 133, 112 and 120 p.c. respectively. Alkaline sodium phosphate causes elongation. Thus the lengths of 6 pieces of dog's stomach muscle immersed in isotonic disodium hydrogen phosphate (0·056 *M* $Na_2HPO_4$) were 107, 110, 115, 125, 110 and 124 p.c. of original respectively. Neutral phosphate and acid sodium phosphate produce no significant difference. Thus the lengths of 6 pieces of dog's stomach muscle immersed in isotonic phosphate of pH 7 were 96, 92, 104, 108, 100 and 104 p.c. respectively; lengths of another 6 pieces in acid sodium phosphate were 100, 105, 100, 100, 105 and 95 p.c. respectively.

Among the divalent cations, calcium and strontium chlorides cause contraction, and barium chloride elongation. Magnesium chloride causes no significant change. Some of these actions resemble those on frog's muscle. Thus the lengths of 6 pieces of dog's stomach muscle immersed in isotonic calcium chloride solution (0·075 *M* $CaCl_2$) were 63, 85, 63, 63, 59 and 59 p.c. of original respectively. Lengths of 6 pieces in strontium chloride were 60, 60, 67, 67 and 91 p.c. or original respectively. Lengths of 6 pieces in barium chloride were 111, 126, 114, 101, 111 and 111 p.c. of original respectively. In magnesium chloride the lengths were 85, 100, 100, 135, 110 and 75 p.c. of original respectively. Thus the divalent cations cause elongation in the order

$$Ca < Sr < Br.$$

Distilled water and isotonic solution of urea cause active elongation of dog's stomach muscle, as in frog's muscle. Thus the lengths of 6 pieces in distilled water were 169, 156, 125, 125, 122 and 111 p.c. of original respectively. In isotonic urea solution, the lengths were 164, 156, 115, 150, 127 and 150 p.c. of original respectively.

Acetylcholine (1 in $10^4$) causes contraction. Saline of pH 6·5 was used to prevent decomposition. Thus the lengths of 6 pieces of dog's stomach immersed in acetylcholine were 88, 92, 88, 1,000, 65 and 83 p.c. of original respectively. Adrenaline (1 in $10^4$) has no significant action. Thus the lengths of 6 pieces of dog's muscle after 24 hours in adrenaline were 106, 96, 96 100 and 100 p.c. of original respectively.

## Discussion

Some of the substances which cause active elongation of unstriated muscle also cause swelling. But it can be shown that these two are independent processes. For it has been shown on *Mytilus* muscle (Singh, 1939) that there is no relation between swelling and relaxation. A contracted muscle may swell enormously without relaxing. The effect of stretching also shows the same (Singh and Singh, 1950 *b*).

The results on mammalian muscle are not so regular as in frog's muscle, so they are not of so much significance as those of the latter muscle. The action of adrenaline and acetylcholine on frog's muscle is very interesting; in recent years, evidence has been presented by several workers that these two substances are concerned in functions other than that of chemical transmission. It is just possible that chemical transmission is incidental, being an act of nature to kill two birds with one stone, their action through the excitatory mechanism reinforcing their action on the contractile mechanism. These two substances therefore, will be responsible for twitch contraction and twitch inhibition, as they are unstable, and readily destroyed by enzymes. For tonic contraction or tonic inhibition, a permanent substance is required (Singh, 1943; Singh and Singh, 1946). This is possibly some ion.

The action of histamine is interesting as it causes contraction of the contractile mechanism and is known to be liberated during muscular contraction.

The action of thiamine is also interesting so also that of glycine.

## Summary

1. The following substances cause contraction or active elongation of unstriated muscle by direct action on the contractile mechanism.

2. 0·112 *M* NaOH causes active elongation of frog's stomach muscle; the muscle is killed by the alkali. Small concentrations of potassium and magnesium decrease and those of calcium increase the effect of sodium hydroxide.

3. 1 *N* HCl causes contraction of frog's stomach muscle, the muscle being killed in the process. Small concentrations of potassium decrease the effect of hydrochloric acid; magnesium and calcium have the opposite effect. After the muscle is killed by alkali, acid causes contraction. Reimmersion in alkali causes active elongation again. The process can be repeated several times, thus causing contraction and active elongation of dead muscle.

4. Concentrated acids, such as hydrochloric, sulphuric, nitric, phosphoric, lactic, acetic, propionic, butyric cause active elongation of frog's muscle; in phosphoric, sulphuric and nitric acids, the muscle dissolves. Saturated solution of sodium hydroxide causes contraction.

5. Isotonic solutions of other alkalies, potassium hydroxide and sodium carbonate cause active elongation of frog's muscle, the action of sodium carbonate being the most powerful yet found.

6. Isotonic solutions of acids cause active elongation of frog's muscle; these are hydrochloric acid, nitric acid, sulphuric acid, phosphoric acid, lactic acid, acetic acid, propionic acid, butyric acid.

7. Isotonic solutions of some sodium salts cause contraction of frog's muscle by direct action on the contractile mechanism. These are sulphate, oxalate, citrate and acetate. Borate and fluoride cause active elongation.

8. Isotonic solutions of the salts of some heavy metals and protein precipitants cause contraction of frog's muscle by direct action on the contractile mechanism. These are mercuric chloride, zinc sulphate, uranium acetate, sodium tungstate. Trichloracetic acid causes active elongation.

9. Water-soluble vitamins, such as riboflavine and ascorbic acid, have no significant effect on the contractile mechanism of frog's muscle. Thiamine causes contraction.

10. Glycine causes active elongation of frog's muscle by direct action on the contractile mechanism.

11. Urethane causes marked active elongation of frog's muscle; chloralose causes contraction by direct action on the contractile mechanism.

12. Sucrose and glucose cause contraction of frog's muscle by direct action on the contractile mechanism.

13. Histamine causes contraction of frog's muscle by direct action on the contractile mechanism.

14. Adrenaline causes active elongation of frog's muscle by direct action on the contractile mechanism.

15. Small concentration of magnesium increase the effect of sodium chloride and potassium chloride.

16. Varying concentrations of potassium only cause active elongation.

17. The action of various ions on the contractile mechanism of dog's stomach muscle is similar to that on the frog's muscle, but the results are not so regular and hence of less significance than those on frog's muscle.

## References


Singh, I. .. *J. Physiol.*, 1939, **96**, 1.

———— .. *Proc. Ind. Acad. Sci.*, 1943, **18**, 53.

—— and Singh, I. .. *Ibid.*, 1946, **23**, 312.

———— .. *Ibid.*, 1949, **30**, 270.

———— .. *Curr. Sci.*, 1950 *a*, **19**, 60.

———— .. *Proc. Ind. Acad. Sci.*, 1950 *b*. In the Press.

# STUDIES IN THE DROSERACEÆ

## II. A Contribution to the Embryology of Three Species of *Drosera*

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### Introduction

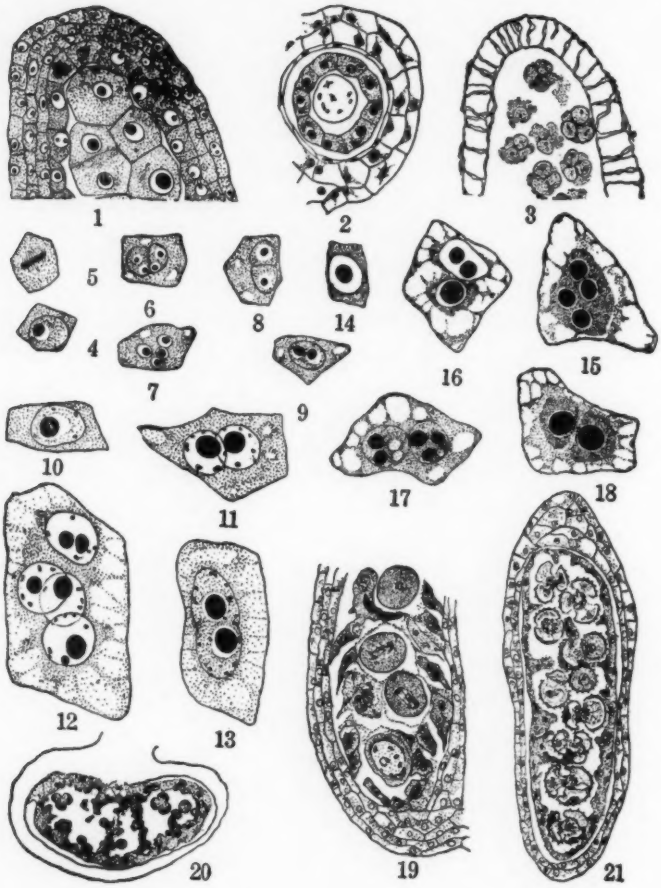
The 90 or more species of *Drosera* are widely distributed throughout the world. Of these the best known, from the embryological point of view, is *D. rotundifolia*. A reference to Schnarf (1929) shows that Peters (1898) was the first to study the embryology of this species. This was followed by the studies of Rosenberg (1899), Lang (1901), Pace (1912) and Souèges (1936). Smith (1929, 1931) has made two important contributions to the morphology of the allied genus *Dionœa* and very recently Narasimhachari (1949) has studied the embryology of *D. Burmanni*. The present paper deals with the embryology of *D. indica* Linn., *D. peltata* Sm., and *D. Burmanni* Vahl.

### Materials and Methods

*D. indica* and *D. Burmanni* were collected from the brink of the Seliur lake, near Tambaram (Madras). *D. peltata*, which is found only at higher elevations, was collected from Ootacamund and Kodaikanal. Very young flower buds were merely trimmed at the apex and dropped into the fixative. In the case of older buds, open flowers and fruits, the ovary was removed, sliced and then plunged into the fixative. Formalin-acetic-alcohol and mercuric chloride were used for fixation; the latter proved better. Sections were cut at a thickness of 8–16 microns and stained in iron-hæmatoxylin. A few preparations were counterstained with orange 'G' in clove oil.

### *The anther*

In all the three species the young anthers (Figs. 1 and 2) show four wall layers, of which the outermost is the epidermis and the innermost the tapetum. In mature anthers, however, the epidermis is found to be shrievelled and the middle layer and tapetum disintegrate so that the only conspicuous layer is the endothecium which develops the usual fibrous thickenings




FIGS. 1–21.—Fig. 1. L.S. anther of *D. Burmanni* showing sporogenous cells and the four wall layers. Note the mitotic divisions in some of the cells of the tapetum (× 1620). Fig. 2. T. S. anther of *D. indica* showing the four wall layers including a prominent tapetum (× 740). Fig. 3. L.S. anther of *D. peltata* to show the fibrous endothecium, traces of epidermis and the microspore tetrads (× 820). Figs. 4 and 5. Uninucleate tapetal cell of *D. Burmanni* showing resting and dividing nucleus (× 3240). Fig. 6. Fusion of tapetal nuclei in pairs (× 1620). Fig. 7. Four nuclei in the tapetal cell before fusion (× 1620). Fig. 8. Two tapetal nuclei in the act of fusion (× 1620). Fig. 9. Result of fusion of four nuclei in a mature tapetal cell (× 1620). Figs. 10, 11 and 12. Uninucleate, binucleate and tetranucleate cells of *D. indica* (× 3240). Fig. 13. Fusion of two nuclei in a tapetal cell; a later stage than Fig. 11 (× 3240). Fig. 14. Uninucleate tapetal cell of *D. peltata* (× 3240). Fig. 15. Four-nucleate tapetal cell; note presence of large vacuoles in the cytoplasm (× 3240). Fig. 16. Tetranucleate stage in which two nuclei have fused and the other two about to fuse; a later stage than Fig. 15. Fig. 17. Tapetal

cell with two tetraploid nuclei. Note the vacuolated condition of the cell and the very thin wall (× 3240). Fig. 18. The two tetraploid nuclei are about to fuse (×3240). Fig. 19. L.S. anther of *D. indica* showing intrusive tapetum (× 740). Fig. 20. L.S. anther of *D. peltata* showing microspore tetrads and false periplasmodium (× 820). Fig. 21. L.S. anther of *D. indica* showing false periplasmodium and microspore tetrads.

(Fig. 3). Dehiscence of the anthers is extrose and occurs by means of longitudinal slits.

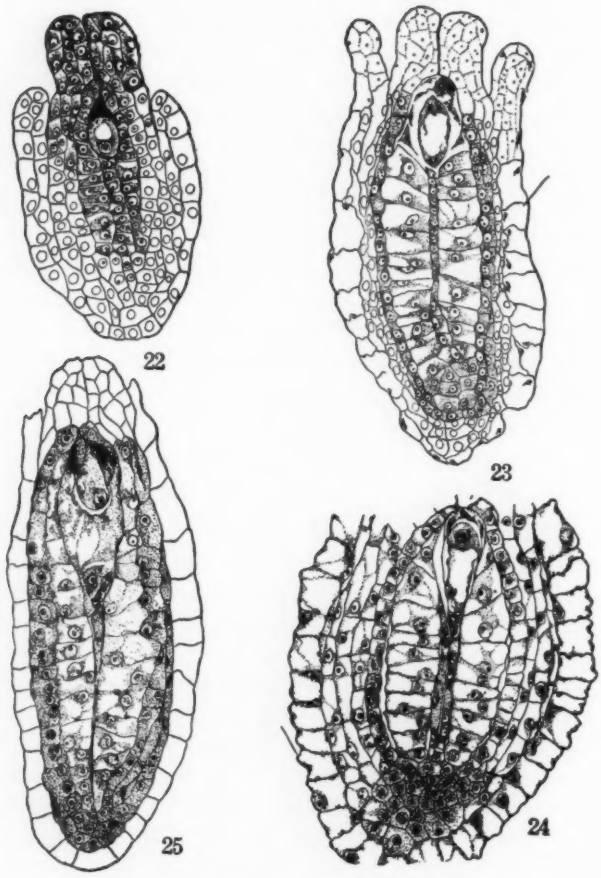
The tapetum, which is of the glandular type, is more or less similar in all the three species. The nucleus of the tapetal cells divides mitotically (Figs. 4–9, 10–13, 14–18), the division generally taking place before the commencement of the meiotic divisions. In *D. indica* and *D. peltata* there is a fusion of the daughter nuclei followed by further nuclear division. During this period, the tapetal cells also show a considerable increase in volume and become highly vacuolated (Figs. 12, 15, 16–18), the vacuoles being somewhat smaller in *D. Burmanni* (Figs. 8 and 9). In *D. Burmanni*, a fusion of the two daughter nuclei formed after the first mitosis is less frequent than in the other two species (Fig. 9). Usually, each of them divides once resulting in four nuclei (Fig. 7). Very soon, however, they fuse in pairs and the two paired nuclei fuse again. The mature tapetal cell, therefore, shows only a single nucleus with two or four nucleoli. In the latter case nuclear fusion is also followed by nucleolar fusion (Figs. 6 and 8). It is to be noted that according to Narasimhachari (1949) the tapetal cells of *D. Burmanni* are binucleate.

In *D. indica* (Figs. 10–13), at the time of tetrad formation, the tapetal cells may be uni-nucleate or binucleate. The former condition is due to the fusion of two nuclei, and the latter to a fusion of four nuclei in pairs.

In *D. peltata* also the behaviour of the tapetal nuclei is more or less similar to the above. The nucleus divides mitotically and the cells become greatly enlarged and vacuolated (Figs. 15 and 16), sometimes obscuring the microspore mother cells. Fig. 17 shows two nuclei within a tapetal cell, each with four prominent nucleoli, suggesting its tetraploid nature. At a later stage the two may fuse to give rise to an octoploid nucleus (Fig. 18). In several instances, particularly in *D. indica* and *D. peltata*, the tapetal cells are found to intrude into the loculus of the anther dividing it into three or more chambers (Figs. 19, 20). Similar instances have been reported by Latter (1926) in *Lathyrus*, Tischler (1927) in *Ribes Gordonianum* and Iyengar (1939) in *Cicer*. These have been discussed by Raghavan and Srinivasan (1942) and by the author (1944). Choudhuri (1942) has noted that in two species of *Limonium*, the pollen sacs sometimes become divided into several chambers, each containing only one pollen mother cell and that these are

in a healthier condition than those developing in anthers without partitions. In *Drosera peltata*, however, the tetrads included in the smaller chambers of the anther usually present a degenerated appearance, probably due to hypernutrition.

During tetrad formation, the tapetal cells of *D. peltata*, and less frequently of *D. indica*, sometimes form a false periplasmodium (Figs. 20, 21). The protoplasts are absorbed by the developing tetrads. In all three species




FIGS. 22–25 (× 740).—**Figs. 22 and 23.** Vertical sections of ovules of *D. peltata* to show the integuments, micropyle and the vacuolated cells of the nucellus. **Fig. 24.** Vertical section of ovule of *D. indica* (for description see text). **Fig. 25.** L.S. ovule of *D. Burmanni* showing the characteristic nucellus and tapetum-like cells of the innermost layer of the inner integument.

the pollen grains remain associated in tetrads. The male gametophytes become three-celled at the shedding stage. In *D. Burmanni* only two cells were observed.

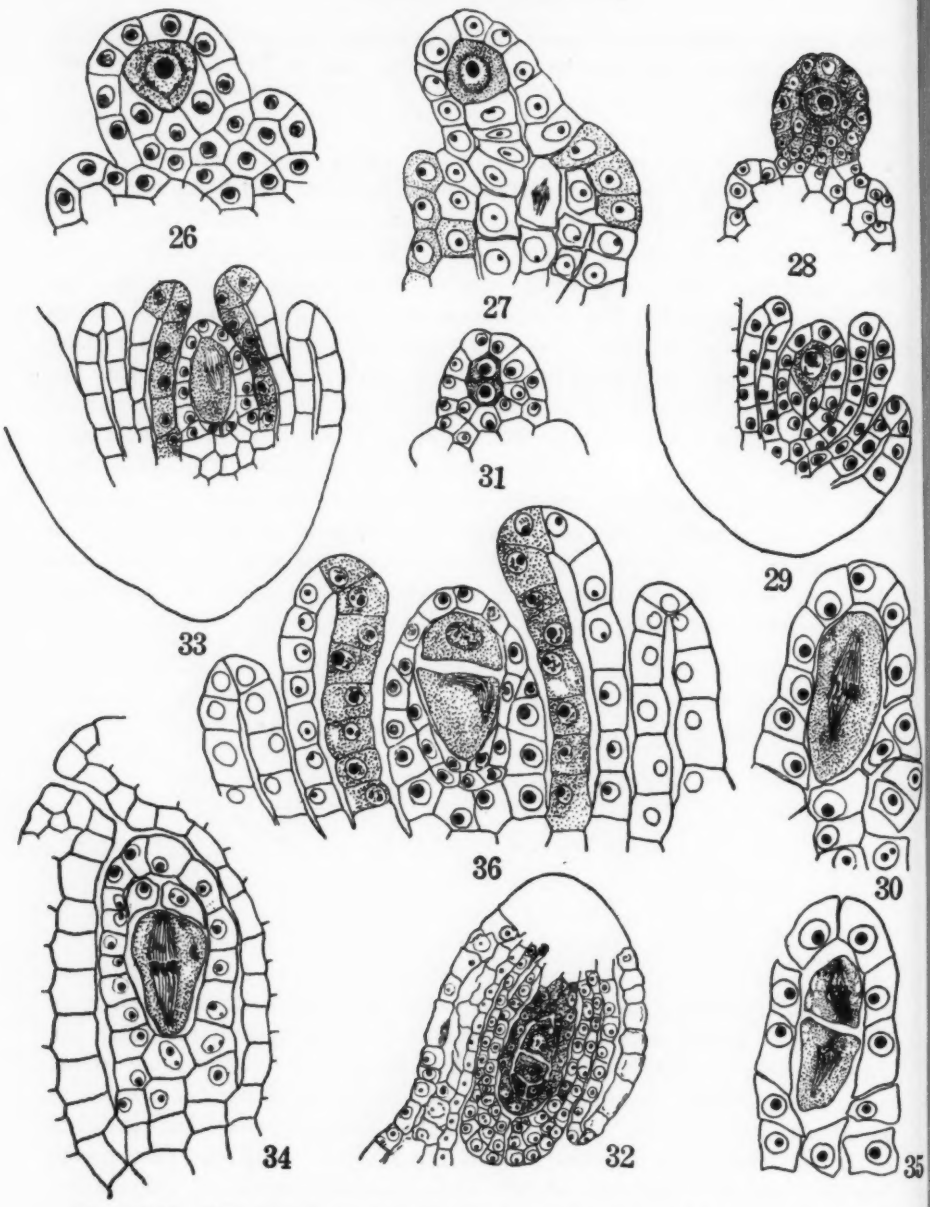
*The ovary and ovule*

The ovary is unilocular and superior. There are numerous anatropous ovules borne on parietal placentas. The number of placentas was found to be three in *D. indica*, two to four in *D. peltata* and four to five in *D. Burmanni*. The young ovule appears as an erect papilla which soon becomes anatropous. The two integuments are each two cells thick (Figs. 22, 32 and 36) but towards the apex the inner integument becomes 4–5 layered (Figs. 22, 32) and it is this part which forms the micropyle. The vascular strand of the ovule terminates near the chalazal region. As reported by Narasimhachari (1949) in *D. Burmanni*, the nucellus is differentiated into two zones (compare Figs. 22–25), one consisting of the large vacuolated cells of the nucellar epidermis and the other consisting of a row of small densely protoplasmic cells, extending from the base of the embryo-sac to the chalaza. At first sight this zone of narrow cells may be mistaken for the conducting strand found in *Casuarina* (Swamy, 1944) or *Drimiopsis* (Rao, 1940); but a careful examination failed to show any tracheids.

Pace (1912) has recorded the presence of air-spaces in the chalazal part of the ovule in *D. rotundifolia*. According to her they form a striking feature of the ovule, but in the three species described here the air-chambers are absent.

*Megasporogenesis*

There is a single hypodermal archesporial cell (Figs. 26, 27 and 28). It differentiates at the time of origin of the inner integument. In *D. indica* (Fig. 29) and *D. peltata* (Fig. 30) the archesporial cell functions directly as the megaspore mother cell after undergoing some enlargement. In *D. Burmanni* it cuts off a parietal cell (Fig. 31) which divides by an anticlinal wall (Figs. 32, 34 and 39). At the dyad stage the two parietal cells can easily be mistaken for the two lower megaspores of a T-shaped tetrad (Fig. 32). The presence of a parietal tissue in *Drosera* has been controverted in the past. Peters (1898) recorded the absence of parietal cells in a species of *Drosera*. On the other hand, Rosenberg (1899) reported their presence in *D. rotundifolia*. Pace (1913) noted the absence of parietal cells except in *D. rotundifolia*. Parietal cells are also formed in *D. Burmanni* (Narasimhachari, 1949) and in the allied genus *Dionæa* (Smith, 1929).




FIGS. 26–35.—Figs. 26–28. Young ovules of *D. indica*, *D. peltata* and *D. Burmanni* showing the hypodermal archesporium and the primordium of the inner integument (× 1620). Fig. 29,

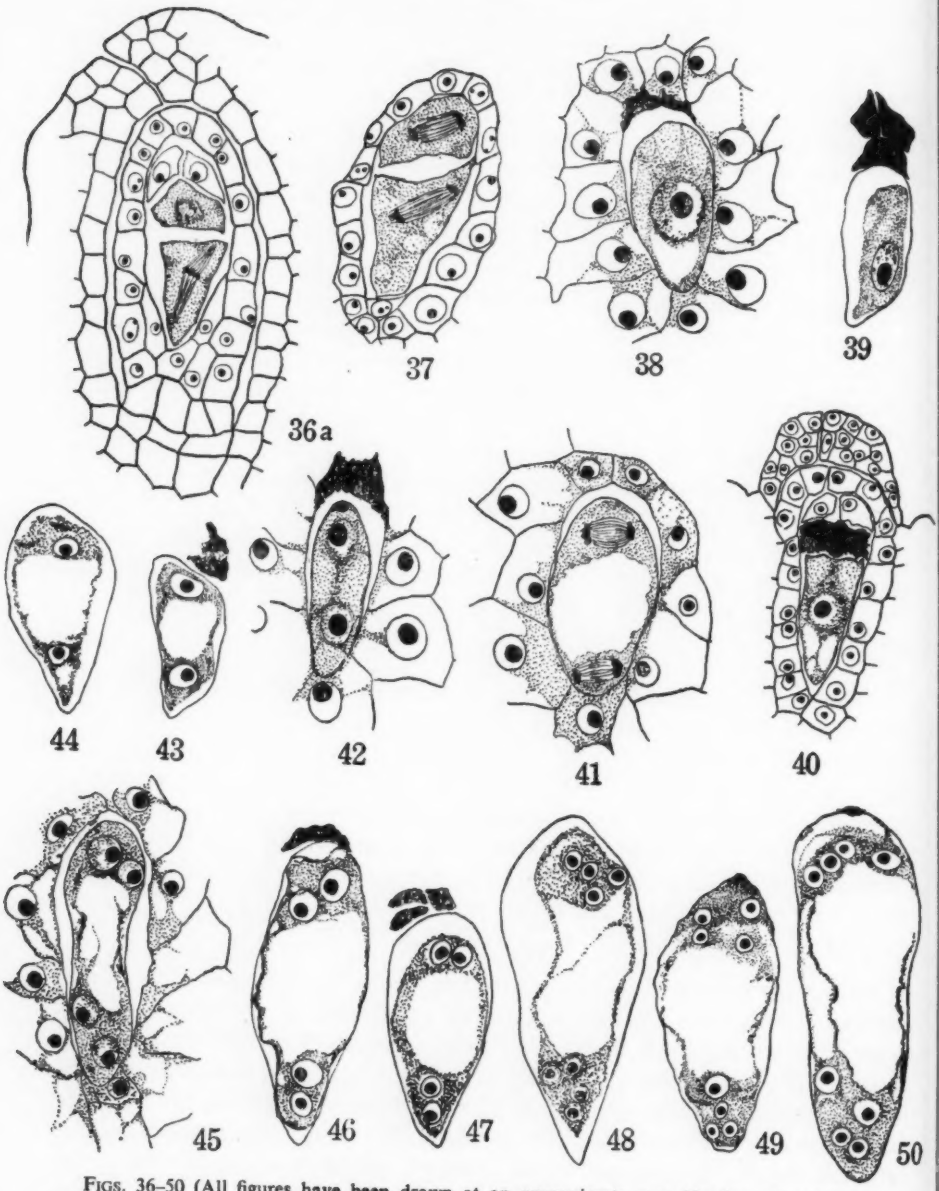
Ovule of *D. indica* showing the two-layered outer and inner integuments and the megaspore mother cell (× 820). Fig. 30. The megaspore mother cell of *D. peltata* undergoing Meiosis I. Note appearance of inner integument (× 1620). Fig. 31. The archesporium of *D. Burmanni* cutting off a parietal cell (×820). Fig. 32. Section of an ovule of *D. Burmanni* showing anticlinal division of primary parietal cell; note the dyad cells (× 1620). Fig. 33. Ovule of *D. indica* showing megaspore mother cell undergoing Meiosis I (× 820). Fig. 34. Megaspore mother cell of *D. Burmanni* in Meiosis I. Note the two parietal cells (× 1620). Fig. 35. Nucellus of *D. peltata* showing dyad cells undergoing Meiosis II (× 1620).

The megaspore mother cell enlarges and undergoes the usual meiotic divisions. Figs. 30, 33 and 34 show the megaspore mother cells of *D. peltata*, *D. indica* and *D. Burmanni* respectively in the metaphase of the first meiotic division. At the end of the first division a cell wall is laid down giving rise to the dyad cells (Figs. 32, 35 and 36). The second division, which may or may not synchronize in both the cells, results in a tetrad of four megaspores (Figs. 35, 36 and 37). The megaspores, as can be anticipated from the disposition of the spindles of meiosis II, are arranged in a slightly zig-zag manner as already pointed out by Rosenberg (1909) and Pace (1912). Narasimhachari (1949) has observed T-shaped tetrads in *D. Burmanni* and I have occasionally come across the same in *D. peltata* (Fig. 39). In all the three species the chalazal megaspore functions and the remaining three degenerate.

*Embryo-sac*

Figs. 38, 39 and 40 show the functioning megaspore in *D. indica*, *D. peltata* and *D. Burmanni* respectively. Vacuoles appear at this stage (Figs. 38, 40) but in one ovule of *D. indica* (Fig. 42) vacuolation was not seen even at the binucleate stage. Other binucleate embryo-sacs showed a large central vacuole with the primary micropylar and primary chalazal nuclei at the poles (Figs. 41, 43 and 44). Figs. 45, 46 and 47 show the 4-nucleate stage. The two nuclei at the micropylar end lie side by side and those at the chalazal end are placed one below the other (Figs. 45, 46 and 47). Figs. 48–50 show the 8-nucleate stage.

The mature embryo-sac is broadest in the middle and tapers at the ends (Figs. 51–54), and occupies about half the length of the nucellus. The synergids are pyriform and a "filiform apparatus" is seen in *D. indica* (Fig. 51) similar to that recorded by Pace (1912) in *D. rotundifolia*. The synergids of *D. peltata* (Figs. 52, 53) have weakly developed hooks. The usual basal vacuole is seen only in *D. indica* (Fig. 51); in *D. peltata* (Fig. 52) the vacuole lies at the apex of the synergid and the nucleus is seen at the base; and in *D. Burmanni* (Fig. 54) although the nucleus lies at the base of the synergid, a vacuole is absent. In *D. indica* (Fig. 51) and *D. Burmanni*




FIGS. 36–50 (All figures have been drawn at an approximate magnification of × 1620).— Figs. 36 and 37. Dyad cells of *D. Burmanni* and *D. indica* undergoing second meiotic division. Note the presence of parietal cells in the former. Figs. 38–40. Functioning megaspore in

*D. indica*, *D. peltata* and *D. Burmanni* respectively. Figs. 41–44. Binucleate embryo-sacs of *D. indica*, *D. peltata* and *D. Burmanni* respectively. Figs. 45–47. Four-nucleate embryo-sacs of *D. indica*, *D. Burmanni* and *D. peltata* respectively. Figs. 48–50. Eight-nucleate embryo-sacs of *D. indica*, *D. peltata* and *D. Burmanni* respectively

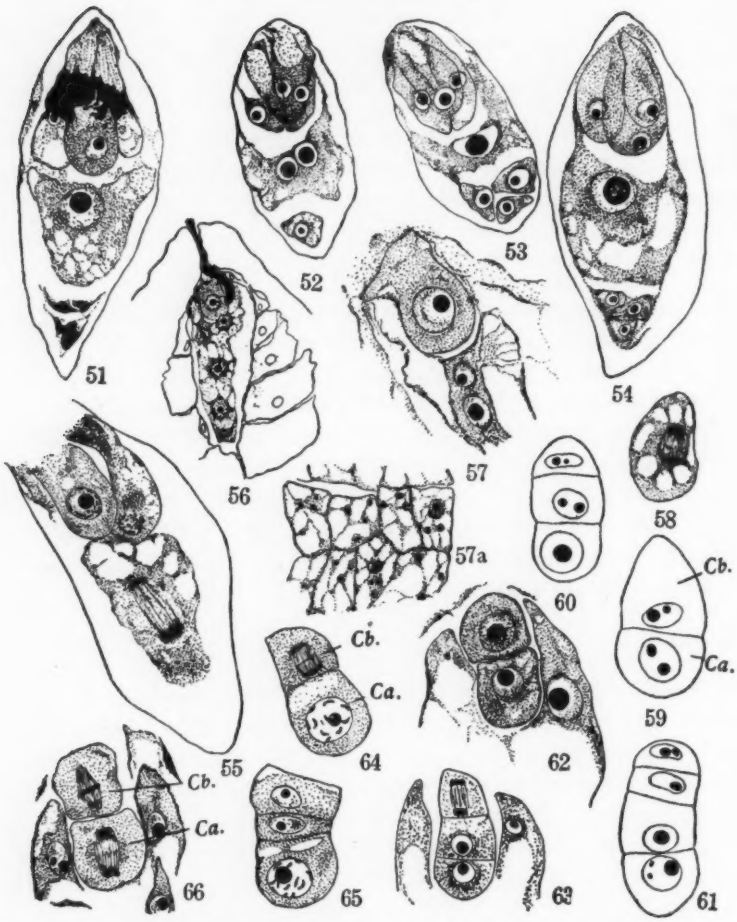
(Fig. 54) the egg is flask-shaped and protrudes a little below the level of the synergids. In *D. peltata* (Figs. 52 and 53), on the other hand, the synergids protrude considerably below the level of the egg. In all cases the nuclei of the synergids and the egg are of the same size and the egg apparatus reaches almost to the middle of the embryo-sac.

The polar nuclei move towards the middle of the embryo-sac and fuse almost immediately. Fig. 52 shows the polar nuclei of *D. peltata* before fusion. These are larger than the rest of the nuclei of the embryo-sac. The secondary nucleus of *D. indica* (Fig. 51) and *D. Burmanni* (Fig. 54) is larger than that of *D. peltata* (Fig. 53). The three nuclei at the chalazal end of the embryo-sac are organized into antipodal cells (Figs. 51, 53 and 54) which are more conspicuous in *D. peltata* and *D. Burmanni* than in *D. indica*. Of the three cells one is basal and the other two are placed laterally. In *D. indica* the antipodals as well as the synergids tend to degenerate earlier than in the other two species.

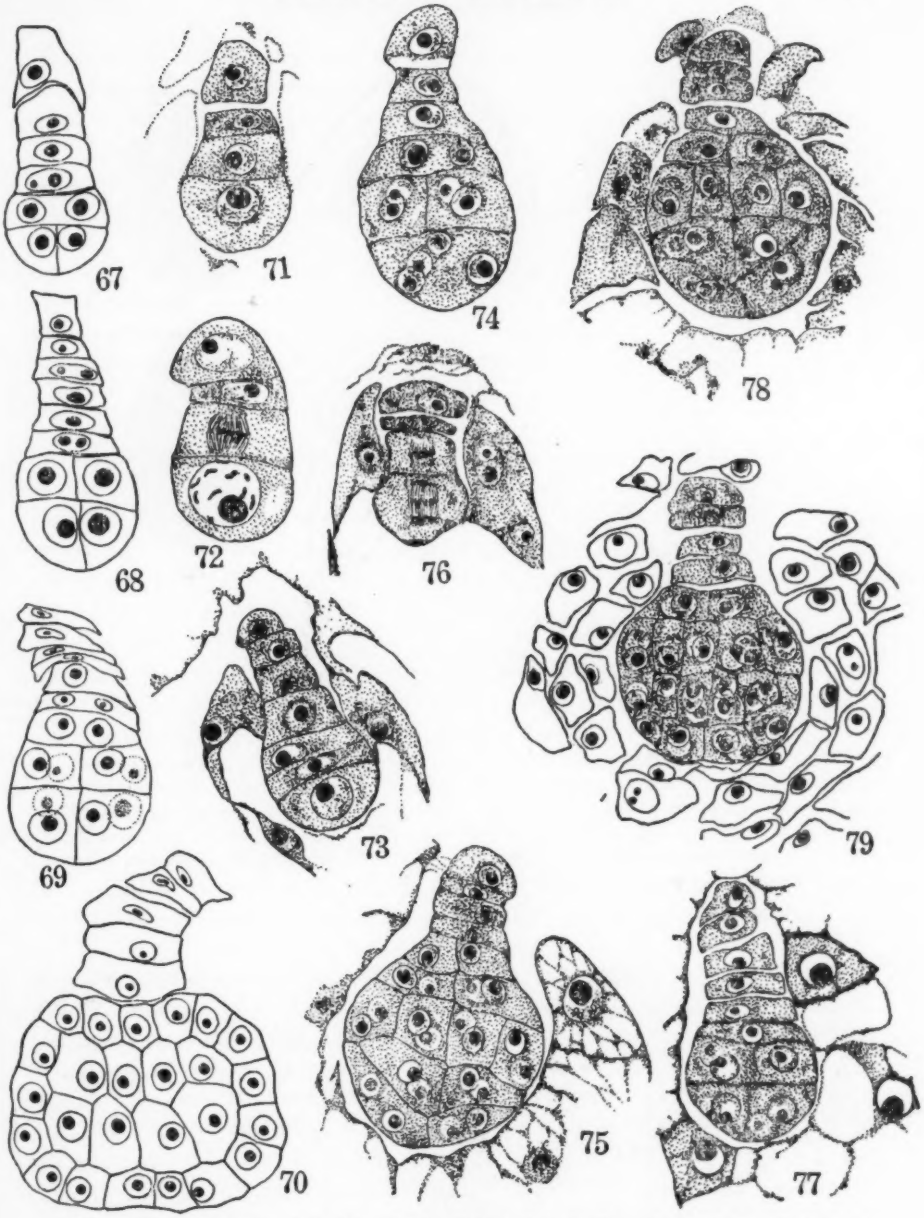
*Endosperm*

Only a few stages relating to fertilization were available. Fig. 55 shows the pollen tube lying in close proximity to the egg. The nucleus inside it is perhaps the vegetative nucleus. The endosperm nucleus is in telophase and the spindle lies parallel to the long axis of the embryo-sac. Fig. 56 shows a later stage showing free nuclear division. From the position of the pollen tube it seems that it penetrates through one of the synergids. Both the synergids disappear at the time of fertilization.

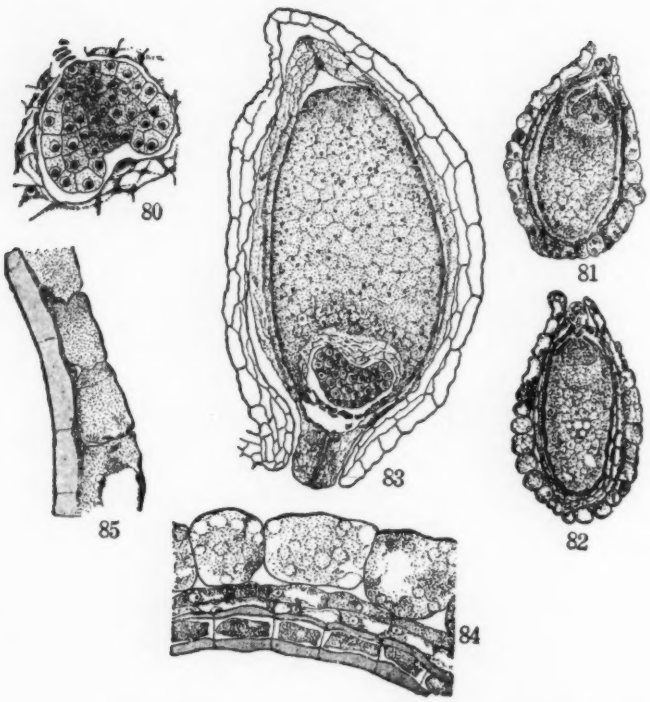
The endosperm nuclei take up a peripheral position in the embryo-sac. A few lie in the vicinity of the zygote and may be mistaken for the nuclei of synergids (Figs. 62, 63 and 75). A greater aggregation of nuclei is seen at the chalazal end of the embryo-sac, while the central part includes a conspicuous vacuole. Wall formation takes place at about the octant stage of the embryo or slightly later (Figs. 77, 78, 79). The endosperm cells were found to contain a number of vacuoles in the early stages (Fig. 57 *a*), but later the cells become packed with starch. In the mature seed the endosperm comprises (1) a zone of hyaline cells with poorly defined walls surrounding the embryo, (2) a large number of polygonal cells packed with starch, and (3) a dense region at the chalazal end.




FIGS. 51-66. (All figures have been drawn at an approximate magnification of × 1620 except Fig. 56 which has been drawn at a magnification of × 820). Fig. 51. Mature embryo-sac of *D. indica*; note synergids with filiform apparatus and the degenerating antipodal cells. Figs. 52 and 53. Mature embryo-sacs of *D. peltata* before and after fusion of polar nuclei. Note the presence of an apical vacuole in one of the synergids (Fig. 52) and a hook (Fig. 53). Fig. 54. Mature embryo-sac of *D. Burmanni*; note the absence of any conspicuous vacuoles in the synergids. Fig. 55. Fertilized embryo-sac of *D. indica* showing division of primary endosperm nucleus. Note pollen tube with a problematical prophasic nucleus at its tip. Fig. 56. Later stage showing free nuclear endosperm. Fig. 57. Zygote in *D. Burmanni*. Fig. 57 *a*. A few young endosperm cells of *D. Burmanni* showing numerous vacuoles which become filled up with starch subsequently. Figs. 58–61. Stages in the development of four-celled proembryo of *D. peltata*. Figs. 62–63. Two and three-celled proembryos of *D. Burmanni*. Note the dividing basal cell. Figs. 64–66. Two- and three-celled proembryos of *D. indica* showing division of basal cell (*cb*) as well as terminal cell (*ca*).




FIGS. 67–79 (× 1620).—Figs. 67 and 68. Octant stage in the embryo of *D. peltata*; note the presence of a suspensor of 4–6 cells. Figs. 69 and 70. Later stages. Figs. 71–75. Stages in the development of embryo in *D. indica*. Figs. 71, 72 and 73. Proembryos showing suspensor. Fig. 74. Octant stage of embryo. Fig. 75. Laying down of periclinal walls in the octants. Fig. 76. A four-celled proembryo of *D. Burmanni* showing simultaneous division in the two apical cells. Fig. 77. Octant stage in *D. Burmanni*. Figs. 78 and 79. Later stages.




FIGS. 80–85.—Fig. 80. Mature embryo of *D. Burmanni* showing the two cotyledonary lobes and the primary histogens (× 1620). Figs. 81 and 82. Vertical sections of mature seeds of *D. indica* and *D. Burmanni*. Note the presence of a small embryo and copious endosperm (× 370). Fig. 83. Vertical section of the seed of *D. peltata* (× 820). Figs. 84 and 85. Part of seed-coat of *D. indica* showing gelatinization of innermost layer and disappearance of the middle layers. Only the outermost layer persists in the mature seed (× 740).

*Embryo*

A few stages in the development of the embryo were met with in *D. peltata*, *D. indica* and *D. Burmanni* (Figs. 58–80). The zygote divides transversely, giving rise to a basal cell (*cb*) and an apical cell (*ca*) (Figs. 58, 59). In *D. indica* (Fig. 64) as well as *D. Burmanni* (Fig. 63) the basal cell (*cb*) divides transversely to give rise to a proembryo of three cells* (Figs. 63 and 65). Actual division of the basal cell was not observed in *D. peltata*; but it is quite probable that here also it divides as in the other two species (Fig. 60). The basal cell divides before the terminal cell (*ca*) in *D. indica*

* According to Narasimhachar (1949) the basal cell does not divide in *D. Burmanni*.

(Figs. 64 and 65) or the two may divide simultaneously (Fig. 66). In *D. Burmanni* (Fig. 63) the basal cell divides only after the terminal cell has completed its division. The 4-celled proembryo, in these species is shown in Figs. 61, 71 and 76. In later stages, a suspensor of 4–7 cells is seen (Figs. 67–77). After the octant stage (Figs. 68 and 77) periclinal divisions take place, in all the tiers of the embryo, to form the dermatogen and other primary histogens (Figs. 75, 78 and 79). Figs. 81, 82 and 83 represent median longitudinal sections of the mature seed of *D. indica*, *D. Burmanni* and *D. peltata* respectively. The embryo of *D. Burmanni* is enlarged and shown in Fig. 80. As can be seen from these figures, the embryo is very small compared to the endosperm.

*Fruit and seed*

The fruit is a capsule containing a large number of small shiny seeds with a rugose wall. The micropylar region of the seed is much narrower than the chalazal and can be easily identified. The seed-coat is very similar in *D. indica* (Fig. 81) and *D. Burmanni* (Fig. 82). In early stages the four layers of the integuments are easily identifiable (Fig. 84). The outermost layer becomes much enlarged and thickened, the next two layers become squeezed and crushed (Fig. 85) and the innermost layer is gelatinized and absorbed. In the mature seed, therefore, the seed-coat comprises only one layer of cells which can be traced back to the outermost layer of the outer integument. This becomes very hard and tough.

## Discussion

The development of pollen grains in *D. indica*, *D. Burmanni* and *D. peltata* is essentially similar. The microspore mother cells and tetrads are nourished by the glandular tapetum which in some cases become intrusive thereby establishing a better contact with the sporogenous cells. The chambering of the anther loculus, which occur occasionally in *D. indica* and *D. peltata*, and the formation of a false periplasmodium may be interpreted as auxiliary devices towards a better nourishment of the microspore mother cells. The pollen grains in these species and in the allied genus *Dionæa* (Smith, 1929) remain in tetrads.

The young ovule is built on the same plan in all three species. For example, the presence of two integuments arising from the base of the nucellus, the nature of the micropyle and the absence or poor development of parietal tissue are a few of the common features. The nucellus consists of radially elongated cells with prominent vacuoles. According to Pace (1912) such vacuolated cells and the air chambers formed in the chalazal

part of the ovule of *D. rotundifolia* enable the seed to float in water. But Smith (1929), with reference to *Dionæa muscipula*, contends that the buoyancy of the seed in water cannot be due to the vacuolated nucellar cells as there is no trace of the nucellus in the seed. In *Drosera* the seeds of all three species sink at once in water.

The nucellar cells might more probably be concerned with the nutrition of the female gametophyte until the fomation of the endosperm. In some ways they are very similar to the cells of the anther tapetum.

There is also the possibility of these cells storing up air for respiration; that is the nucellus acts as a gas vacuole which may also help the ovule to withstand dessication. These species of *Drosera* have been found to grow in marshy habitat and therefore a device for storage of air could be expected. In *D. rotundifolia* air chambers are formed in the chalazal part of the ovule (Pace, 1912) although these are not found in the three species investigated. The lack of air chambers, in these species need not be prejudicial if the nucellar epidermis can serve the same purpose.

## Summary

1. The development of the anther, ovule, famale gametophyte and embryo have been traced in *D. indica*, *D. peltata* and *D. Burmanni*.

2. The anther wall consists of four layers—epidermis, a fibrous endothecium, an ephemeral middle layer and the tapetum. Pollen grains remain in tetrads. They have varying numbers of specialized germ pores. The tapetum is glandular and consists of tetraploid or octoploid cells. Occasionally it intrudes into the anther locules dividing it into chambers. A false periplasmodium is found occasionally in *D. peltata* and *D. indica*.

3. Ovules are bitegumic; the inner integument forms the micropyle. The nucellus consists vacuolated cells; the possible function of these has been discussed. The archesporium is hypodermal and one-celled. It cuts off a parietal cell only in *D. Burmanni;* in the other two species it directly functions as the megaspore mother cell. The chalazal megaspore functions. The embryo-sac conforms to the monosporic eight-nucleate type. A 'filiform apparatus' is seen in the synergids of *D. indica*. Triple fusion precedes syngamy.

4. The primary endosperm nucleus undergoes a number of free nuclear divisions before the wall is laid down. The proembryo shows a suspensor of 4–7 cells. The mature embryo is dicotyledonous and is embedded in the endosperm. The seed is protected by one layer of cells.

ACKNOWLEDGEMENTS

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REFERENCES

Banerji, I. .. "Sterility in *Colocasia antiquorum* Schott," *Jour. Ind. Bot. Soc.*, 1937, **16**, 159–64.

Choudhuri, H. C. .. "Chromosome studies in some British species of *Limonium*," *Ann. Bot.*, 1942, N.S., **6**, 183–217.

Iyengar, N. K. .. "Cytological investigations on the genus *Cicer*," *Ann. Bot.*, 1939, N.S. **3**, 271–305.

*Lang, F. X. .. "Untersuchungen über Morphologie, Anatomie und Samenentwicklung von *Polypompholyx* und *Byblis gigantea*," *Flora*, 1901, **88**, 149–206.

Latter, J. .. "The pollen development of *Lathyrus odoratus*," *Ann. Bot.*, 1926, **40**, 277–313.

Maheshwari, P. .. "Recent work on the types of embryo-sacs in Angiosperms—A critical review," *Jour. Ind. Bot. Soc.*, 1941, **20**, 229–61.

Murthy, K. L. .. "Gametogenesis and embryogeny in some Commelinaceæ," *Ibid.*, 1938, **17**, 101–16.

Narasimhachar, S. G. .. "A contribution to the embryology of *Drosera Burmanni* Vahl.," *Proc. Ind. Acad. Sci.*, 1949, **B, 29**, 98–104.

Pace, L. .. "*Parnassia* and some allied genera," *Bot. Gaz.*, 1912, **54**, 306–29.

*Peters, C. A. .. "Reproductive organs and embryology of *Drosera*," *Proc. Amer. Ass. Adv. Sci.*, 1898, **46**, 275.

Rao, Y. S. .. "Structure and development of the embryo-sac of *Drimiopsis Kirki* Baker and *Allium Govanianum* Wall.," *Jour. Ind. Bot. Soc.*, 1940, **19**, 273–82.

Rosenberg, O. .. "Physiologisch-cytologische Untersuchungen über *Drosera rotundifolia* Upsala," 1899.

——— .. "Cytologische und morphologische Studien an *Drosera longifolia* × *rotundifolia*," *Kungl Svenska Vet Akad. Handl.*, 1908, **43**, (11), 1–64.

Raghavan, T. S. and Srinivasan, V. K. .. "A contribution to the life-history of *Vahlia viscosa* Roxb. and *Vahlia oldenlandioides* Roxb.," *Proc. Ind. Acad. Sci.*, 1942, **B 15**, 83–105.

* These references were not available to me in original.

Schnarf, K. .. *Embryologie der angiospermen*, Berlin, 1929.

Smith, C. M. .. "Development of *Dionæa Muscipula*. I. Flower and seed," *Bot. Gaz.*, 1929, **87**, 507–30.

———— .. "Development of *Dionæa Muscipula*. II. Germination of seed and development of seedling to maturity," *Ibid.*, 1931, **91**, 377–93.

Souéges, R. .. "Embryogénie des Droseracées Developement de l'embryon chez le *Drosera rotundifolia* L.," *Compt. Rend. Acad. Sci.*, Paris, 1936, **11**, 1457–59.

Swamy, B. G. L. .. "A preliminary note on the embryology of *Casuarina equisetifolia* Forst.," *Proc. Ind. Acad. Sci.*, 1944, **B 20**, 187–91

Tischler, G. .. "Chromosomenstudien bei *Ribes Gordonianum* und seinen Eltern," *Planta*, 1927, **4**, 617–50.

Venkatasubban, K. R. .. *Cytological Studies in Bignoniaceæ*, Diss., Annamalai University, 1944.

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The development of pollen grains in *D. indica*, *D. Burmanni* and *D. peltata* is essentially similar. The microspore mother cells and tetrads are nourished by the glandular tapetum which in some cases become intrusive thereby establishing a better contact with the sporogenous cells. The chambering of the anther loculus, which occur occasionally in *D. indica* and *D. peltata*, and the formation of a false periplasmodium may be interpreted as auxiliary devices towards a better nourishment of the microspore mother cells. The pollen grains in these species and in the allied genus *Dionæa* (Smith, 1929) remain in tetrads.

The young ovule is built on the same plan in all three species. For example, the presence of two integuments arising from the base of the nucellus, the nature of the micropyle and the absence or poor development of parietal tissue are a few of the common features. The nucellus consists of radially elongated cells with prominent vacuoles. According to Pace (1912) such vacuolated cells and the air chambers formed in the chalazal

part of the ovule of *D. rotundifolia* enable the seed to float in water. But Smith (1929), with reference to *Dionæa muscipula*, contends that the buoyancy of the seed in water cannot be due to the vacuolated nucellar cells as there is no trace of the nucellus in the seed. In *Drosera* the seeds of all three species sink at once in water.

The nucellar cells might more probably be concerned with the nutrition of the female gametophyte until the fomation of the endosperm. In some ways they are very similar to the cells of the anther tapetum.

There is also the possibility of these cells storing up air for respiration; that is the nucellus acts as a gas vacuole which may also help the ovule to withstand dessication. These species of *Drosera* have been found to grow in marshy habitat and therefore a device for storage of air could be expected. In *D. rotundifolia* air chambers are formed in the chalazal part of the ovule (Pace, 1912) although these are not found in the three species investigated. The lack of air chambers, in these species need not be prejudicial if the nucellar epidermis can serve the same purpose.

## Summary

1. The development of the anther, ovule, famale gametophyte and embryo have been traced in *D. indica*, *D. peltata* and *D. Burmanni*.

2. The anther wall consists of four layers—epidermis, a fibrous endothecium, an ephemeral middle layer and the tapetum. Pollen grains remain in tetrads. They have varying numbers of specialized germ pores. The tapetum is glandular and consists of tetraploid or octoploid cells. Occasionally it intrudes into the anther locules dividing it into chambers. A false periplasmodium is found occasionally in *D. peltata* and *D. indica*.

3. Ovules are bitegumic; the inner integument forms the micropyle. The nucellus consists vacuolated cells; the possible function of these has been discussed. The archesporium is hypodermal and one-celled. It cuts off a parietal cell only in *D. Burmanni;* in the other two species it directly functions as the megaspore mother cell. The chalazal megaspore functions. The embryo-sac conforms to the monosporic eight-nucleate type. A 'filiform apparatus' is seen in the synergids of *D. indica*. Triple fusion precedes syngamy.

4. The primary endosperm nucleus undergoes a number of free nuclear divisions before the wall is laid down. The proembryo shows a suspensor of 4–7 cells. The mature embryo is dicotyledonous and is embedded in the endosperm. The seed is protected by one layer of cells.

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REFERENCES

Banerji, I. .. "Sterility in *Colocasia antiquorum* Schott," *Jour. Ind. Bot. Soc.*, 1937, **16**, 159–64.

Choudhuri, H. C. .. "Chromosome studies in some British species of *Limonium*," *Ann. Bot.*, 1942, N.S., **6**, 183–217.

Iyengar, N. K. .. "Cytological investigations on the genus *Cicer*," *Ann. Bot.*, 1939, N.S. **3**, 271–305.

*Lang, F. X. .. "Untersuchungen über Morphologie, Anatomie und Samenentwicklung von *Polypompholyx* und *Byblis gigantea*," *Flora*, 1901, **88**, 149–206.

Latter, J. .. "The pollen development of *Lathyrus odoratus*," *Ann. Bot.*, 1926, **40**, 277–313.

Maheshwari, P. .. "Recent work on the types of embryo-sacs in Angiosperms—A critical review," *Jour. Ind. Bot. Soc.*, 1941, **20**, 229–61.

Murthy, K. L. .. "Gametogenesis and embryogeny in some Commelinaceæ," *Ibid.*, 1938, **17**, 101–16.

Narasimhachar, S. G. .. "A contribution to the embryology of *Drosera Burmanni* Vahl.," *Proc. Ind. Acad. Sci.*, 1949, **B, 29**, 98–104.

Pace, L. .. "*Parnassia* and some allied genera," *Bot. Gaz.*, 1912, **54**, 306–29.

*Peters, C. A. .. "Reproductive organs and embryology of *Drosera*," *Proc. Amer. Ass. Adv. Sci.*, 1898, **46**, 275.

Rao, Y. S. .. "Structure and development of the embryo-sac of *Drimiopsis Kirki* Baker and *Allium Govanianum* Wall.," *Jour. Ind. Bot. Soc.*, 1940, **19**, 273–82.

Rosenberg, O. .. "Physiologisch-cytologische Untersuchungen über *Drosera rotundifolia* Upsala," 1899.

———— .. "Cytologische und morphologische Studien an *Drosera longifolia* × *rotundifolia*," *Kungl Svenska Vet Akad. Handl.*, 1908, **43**, (11), 1–64.

Raghavan, T. S. and Srinivasan, V. K. .. "A contribution to the life-history of *Vahlia viscosa* Roxb. and *Vahlia oldenlandioides* Roxb.," *Proc. Ind. Acad. Sci.*, 1942, **B 15**, 83–105.

* These references were not available to me in original.

Schnarf, K. .. *Embryologie der angiospermen*, Berlin, 1929.

Smith, C. M. .. "Development of *Dionæa Muscipula*. I. Flower and seed," *Bot. Gaz.*, 1929, **87**, 507–30.

———— .. "Development of *Dionæa Muscipula*. II. Germination of seed and development of seedling to maturity," *Ibid.*, 1931, **91**, 377–93.

Souéges, R. .. "Embryogénie des Droseracées Developement de l'embryon chez le *Drosera rotundifolia* L.," *Compt. Rend. Acad. Sci.*, Paris, 1936, **11**, 1457–59.

Swamy, B. G. L. .. "A preliminary note on the embryology of *Casuarina equisetifolia* Forst.," *Proc. Ind. Acad. Sci.*, 1944, **B 20**, 187–91

Tischler, G. .. "Chromosomenstudien bei *Ribes Gordonianum* und seinen Eltern," *Planta*, 1927, **4**, 617–50.

Venkatasubban, K. R. .. *Cytological Studies in Bignoniaceæ*, Diss., Annamalai University, 1944.

# THE FOOD OF SOME YOUNG CLUPEIDS*

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## Introduction

The problem of the food of fishes first attracted the attention of a few ichthyologists towards the end of the last century, and since then several investigations have been carried out all over the world. The beginning of such a type of study was first made by Day (1882), who investigated the food of the herring. The pioneer work in this direction was undertaken by Herdmann (1892) in Europe on the food of *Solea vulgaris* and *Pleuronectes platessa*. Later he investigated the problem of food of young fishes in collaboration with Andrew Scott. The latter (1902) studied the food habits of the herring and some other fishes while working with the Scotland Fisheries Board. Johnstone (1906) gave a comparative account of the food habits of the plaice and dab. Correlation between the plankton and the mackerel fishery was found by Bullen (1908), who worked out the feeding habits of the mackerel and some Clupeids in the English Channel. Hardy (1924) recorded the feeding habits of herring of the East coast of England, while Steven (1930) studied the bottom fauna and its bearing on the food of fishes.

The credit of developing the problem further and giving it the important place that it occupies to-day in fishery research goes to Lebour (1918–34), who published a series of papers on the food and feeding habits of the larval and post-larval fishes of the fishery grounds off Plymouth. She has done a good deal of work on the food of Clupeids and has conclusively shown the changes of food habits at different stages of their life-history.

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In spite of the great importance in India of the study of the food of fishes, very little is known about the food habits of our common marine fishes, except for a few notes on the stomach contents of some forms. Hornell and Naidu (1923) and Devanesan (1942) have worked on the food of *Sardinella longiceps* and *S. gibbosa* respectively and its relation to the incidence of plankton in Indian waters. A fairly comprehensive work, dealing with the feeding habits of the Perches of the Madras coast by Job (1940), Gopinath's (1942) brief account of the distribution and feeding habits of some post-larval fishes of the Trivandrum coast and the papers of Chidambaram (1944), Chacko (1944, 1945, 1946 and 1949) and Venkatraman (1944) are among those dealing with the feeding habits of Indian food-fishes.

Except for a passing mention of the food habits of *Harpodon nehereus* (Hora, 1924) practically nothing is known about the food habits of the fishes of the Bombay waters. The present investigation has been undertaken to fill up the gaps in this respect.

## Material and Methods

The material consisting of fish larvæ, post-larvæ and immature fishes was brought to the laboratory from various centres around Bombay and also from plankton samples taken twice a week from Apollo Bunder, Choupati, Mahim Creek and Versova during the period, June 1945 to June 1947. 473 Plankton samples in all were taken with an ordinary surface tow-net in the course of these studies. Two hauls, each of fifteen minutes duration, were generally taken each time, making a total of four samples in a week. The fish larvæ and post-larvæ were sorted out for the examination of stomach contents and the rest of the plankton was fixed in 5% sea water formalin.

To study more fully the food habits of a fish in different stages, each species was divided for the sake of convenience into the following four stages according to the size of the specimens. They do not, however, denote the growth stages and are purely arbitrary. Specimens ranging from 1–25 mm. were put in stage I, those between 25–50 mm. in stage II, between 50–75 mm. in stage III and those 75 and above upto 100 mm. in stage IV. Variations, if any, in the food habits of a species, according to the seasons in relation to the availability of plankton were also noted wherever possible. For the sake of convenience the calendar year has been divided into four seasons, namely, the Rainy season—16th June to 30th September, the Dry season—October to November, the Winter season—December to February and the Summer season—March to 15th June.

Altogether 1,706 young specimens of fish were obtained from the fishermen's catch and plankton samples. The specimens from the plankton were killed and dissected immediately and those from the fishermen's catch in as fresh a condition as possible. The stomach contents were then removed and examined immediately as far as possible and preserved in 5% sea water formalin for further critical study. Identification of the stomach contents could not be done thoroughly mainly due to the small size of the specimens. The stomachs of majority of fishes contained mostly food of the same variety, and this condition was recorded as 100%. In cases where different types of food material were observed, the percentages were noted against them by simple eye estimation for every individual specimen, as no other method was feasible, taking into consideration the small size of the specimens studied. Then all the estimated percentages of specimens of a species of one stage were tabulated and the total percentage for that stage was arrived at by the method of weighted frequencies.

## Occurrence of Zooplankton in Bombay Waters during 1945–1947

A precise knowledge of the occurrence, abundance and seasonal fluctuation of plankton of a place is essential in studying the food habits of a number of animals of that area. So a study of the occurrence of plankton in local waters in order to determine the food-habits of our fishes by examining their stomach contents and correlating these results with the occurrence of plankton was simultaneously undertaken.

The general occurrence, abundance and seasonal fluctuations of the major groups in the zooplankton of Bombay Harbour has been studied by Bal and Pradhan (1945) and of phytoplankton by Gonzalves (1947). Our observations deal with the occurrence of only such planktonic forms as generally form the principal items of food or the bulk of the food of young fishes. The various groups constituting such food are the following:—

*Copepods.*—The copepods form one of the major groups of planktonic animals and are very common in tow-net samples. It has been observed that copepods in Bombay Harbour have two maxima or peak periods once in January and again in July. They were found in fairly large numbers during the rainy and winter seasons, while their number had fallen considerably in August and November. The more common species found were *Euchæta suberassus* (Giesbrecht), *Acrocalanus monachus* (Giesbrecht) and *Euchæta marina* (Giesbrecht).

*Prawn larvæ.*—These form an important group of our plankton, occurring in large numbers practically throughout the year. Many a time the

entire catch was made up of these larvæ in their various developmental stages. They were predominant in the rainy and early winter seasons. The common prawn larvæ recorded belonged to the genus *Acetes*.

*Other crustacean larvæ.*—(*a*) Copepod larvæ appeared throughout the year, their maximum occurrence being in the month of December.

(*b*) Stomatopod larvæ occurred in small numbers during the dry and summer seasons.

(*c*) Zoea of *Porcellana* was obtained throughout the year except in the rainy season, swarming at intervals in December.

(*d*) Zoea of *Brachyura* was collected during the winter and summer seasons, the peak period of occurrence being in the month of March.

(*e*) Megalopa occurred in small numbers throughout the year, occasionally swarming in May.

(*f*) Phyllosoma of *Panilurus* was recorded in small numbers in the winter season only.

(*g*) Lucifers occurred in fairly good numbers throughout the year.

*Polychæta.*—*Tomopteris* were encountered in small numbers all the year round.

## General Observations

The Clupeids popularly known as "Herrings", "Sardines", "Pilchards", "Sprats" and the "Anchovies" form an important group of edible fishes, whose post-larval and immature forms abound in the waters around Bombay. The members of this group are represented in this collection by the following sixteen species belonging to nine genera and two families:—

[1]Family—*Clupeidæ*.

Subfamily—*Dorosomatini*.

Genus *Nematolosa* Regan.

Species. *Nematolosa nasus* (Bl.).

Subfamily—*Clupeini*.

Genus *Harengula* Cuvier & Valenciennes.

Species *Harengula punctata* (Rüpp.).

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[1] Misra, K. S., 1947, "A check list of fishes of India, Burma and Ceylon. II. Clupeiformes, Bathyclupeiformes, Galaxiiformes, Scopeliformes and Atcleopiformes," *Rec. Indian Mus.*, **45**, Pt. IV, 377–431.

Genus *Sardinella* Cuvier & Valenciennes.
Species *Sardinella albella* (C.V.).

Genus *Hilsa* Regan.
Species *Hilsa toli* (C.V.).

Genus *Kowala* Cuvier & Valenciennes.
Species *Kowala coval* (C.).

Genus *Ilisha* Richardson
Species *Ilisha brachysoma* (Blkr.).
*Ilisha motius* (Ham.).
*Ilisha filigera* (C.V.).
*Ilisha elongata* (Benn.).

Family—*Engraulidæ*.

Genus *Thrissocles* Jordan and Evermann
Species. *Thrissocles kammalensis* (Blkr.).
*Thrissocles hamiltonii* (Gray.).
*Thrissocles purava* (Ham.).
*Thrissocles dussumieri* (C.V.).

Genus *Anchoviella* Fowler
Species *Anchoviella commersonii* (Lac.).
*Anchoviella tri* (Blkr.).

Genus *Coilia* Gray
Species *Coilia dussumieri* (C.V.).

The larvæ of *Thrissocles kammalensis* were encountered only in plankton samples, whereas those of *Hilsa toli*, *Ilisha brachysoma*, *I. motius*, *Thrissocles purava*, *T. dussumieri* and *Anchoviella commersonii* were obtained both from plankton and fisermen's catches. The post-larval forms of *Sardinella albella*, *Harengula punctata*, *Ilisha elongata*, *I. filigera*, *Nematolosa nasus*, *Thrissocles hamiltonii*, *Anchoviella tri* and *Coilia dussumieri* were found exclusively in fishermen's collections.

In general, the young forms of this family were found in abundance during the summer and rainy seasons. *Thrissocles kammalensis*, *T. hamiltonii*, *Anchoviella commersonii* and *Coilia dussumieri* occurred practically throughout the year. The larval and post-larval forms of *Hilsa toli*, *Sardinella albella*, *Kowala coval*, *Nematolosa nasus*, *Thrissocles purava*, *T. dussumieri* and all the species of *Ilisha* occurred in good numbers in two or three seasons.

*Harengula punctata* occurred in large numbers only in the dry season and *Anchoviella tri* was collected only once during a period of two years.

Immature forms of these fishes possess high edible qualities and fetch a good price in the local markets. Some species like *Hilsa toli*, *Nematolosa nasus*, *Coilia dussumieri*, *Thrissocles hamiltonii* and *T. purava* attain a size ranging from eight to eighteen inches and are highly esteemed as food. The post-larvæ of most of the species were found in good numbers at Mahim creeks, while their adults along with the immature forms were landed in large numbers, specially in the summer and rainy seasons at Sassoon Docks and Versova.

## Food Habits of Different Species

### *Nematolosa nasus* (Bl.)

Post-larvæ of *N. nasus* occur in large numbers at Mahim creek during the winter and summer seasons in the fishermen's catch, but not in the plankton. This species which is a local food fish, attains a size of 6–7 inches. A total number of 76 post-larval forms, 32–100 mm. in length were examined, 42 in winter and 34 in the summer seasons.

*Number of fish examined and percentage of food taken by each stage*

| Stage of fish | II | III | IV |
|---|---|---|---|
| Total No. of fish examined .. | 46 | 28 | 2 |
| No. of fish with digested food or no food | 37 | 23 | 2 |
| Items of food taken : | % | % | |
| Muddy remains .. | 66·66 | 60·00 | .. |
| Sand and foraminifera .. | 11·11 | 40·00 | .. |
| Brown Algæ .. | 11·11 | .. | .. |
| Copepods .. | 11·11 | .. | .. |

The high percentage of mud and sand in the stomachs of a majority of specimens with food indicates the bottom feeding habits of *N. nasus*. This view is further supported by the fact that they did not feed on the plankton even though it was quite rich during the period of their occurrence. In this connection, it is interesting to note that the stomach of this species is modified into a gizzard-like apparatus, which may be an adaptation to the bottom feeding habits.

### *Harengula punctata* (Rüpp.)

The post-larval forms of *H. punctata* occurred in large numbers in fishermen's catches at Mahim during the winter, but not in any of the plankton samples. It is locally called as " Kanat " and attains a size of 6–7 inches.

When it occurs in large quantities, it is sun-dried and sent to interior places for consumption. In all 43 specimens ranging from 51–87 mm. were collected from Mahim creeks, but earlier forms were not available.

*Number of fish examined and percentage of food taken by each stage*

| Stage of fish | III | IV |
|---|---|---|
| Total No. of fish examined .. | 38 | 5 |
| No. of fish with digested or no food | 19 | 2 |
| Items of food taken : | % | % |
| Muddy remains .. | 47·37 | .. |
| Sandy remains .. | 26·33 | .. |
| Gastropods .. | 10·52 | .. |
| Prawn larvæ .. | 10·52 | 66·66 |
| Copepods .. | 5·26 | 33·33 |

The presence of a high percentage of mud and sand in the stomachs tends to show that *H. punctata* is probably a bottom feeder. It may be utilising the organic food material taken along with the sand and mud from the bottom of the sea.

*Sardinella albella* (C.V.)

Post-larval and immature forms of *S. albella* were found in fishermen's catch at Sassoon Docks, Mahim and Versova, but were absent in plankton samples. In all 49 specimens ranging from 26–51 mm. were collected, 8 in dry, 31 in winter and 10 in the summer seasons and all of them were placed in the second stage. It is stated that *S. albella* attains a size of about 8 inches, but specimens of this size were not found by us at Bombay.

*Number of fishes examined and percentage of food taken by each stage*

| Stage of fish | II |
|---|---|
| Total No. of fish examined .. | 49 |
| No. of fish with digested or no food | 37 |
| Items of food taken : | % |
| Copepods .. | 66·66 |
| Prawn larvæ .. | 16·66 |
| Sand and foraminifera .. | 8·33 |
| Crustacean remains .. | 8·33 |

The precise nature of the food of this species cannot be stated with the data on hand, particularly when the stomachs of a large number of speci-

mens were without food. However, copepods ranked the first according to percentage basis indicating plankton feeding habits.

*Hilsa toli* (C.V.)

Post-larval and immature forms of *H. toli* are very common in Bombay waters particularly in the summer and rainy seasons. The adult fish, popularly known as " Bhing ", usually attains a size of 2–3 feet in length and is a favourite table fish. A total number of 169 specimens, 66 in rainy, 32 in winter and 71 in the summer seasons were collected from fishermen's catches at Sassoon Docks and Mahim and also from plankton samples. A record regarding their food habits is given below.

*Number of fish examined and percentage of food taken by each stage*

| Stage of fish | I | II | III | IV |
|---|---|---|---|---|
| Total No. of fish examined .. | 13 | 56 | 78 | 22 |
| No. of fish with digested or no food | 8 | 40 | 37 | 7 |
| Items of food taken : | % | % | % | % |
| Diatoms .. | 100 | 31·25 | 46·34 | 33·33 |
| Copepods .. | .. | 50·00 | 43·90 | 60·00 |
| Megalopa .. | .. | 12·50 | .. | .. |
| Polychætes . | .. | 6·25 | .. | .. |
| Prawn larvæ .. | .. | .. | 2·43 | .. |
| Muddy remains .. | .. | .. | 7·31 | 6·66 |

From the available data *H. toli* can be classed amongst the omnivorous plankton feeders as diatoms were taken in appreciable numbers along with copepods. Diatoms mostly belonging to the genus *Coscinodiscus* were taken more during the summer season. The presence of mud in the stomachs of the later stages gives an indication of its bottom feeding habits.

Chacko (1949) who has examined specimens varying from 180 to 240 mm. states that they feed mainly on macroplankton, consisting of prawns, zoea and stomatopod larvæ.

*Kowala coval* (C.)

Post-larval and immature forms of *K. coval*, locally known as " Bhitgi " were common throughout the year, except the rainy season and were found both in plankton and fishermen's catches. The maximum size of this species recorded by Day is 4 inches. In all 101 specimens, measuring 19–63 mm, were collected, 20 in dry, 36 in winter and 45 in the summer seasons. The results of the examination of their stomach contents are given below.

*Number of fish examined and percentage of food taken by each stage*

| Stage of fish | I | II | III |
|---|---|---|---|
| Total No. of fish examined .. | 19 | 69 | 13 |
| No. of fish with digested or no food | 14 | 52 | 10 |
| Items of food taken : | % | % | % |
| Copepods .. | 80·00 | 47·05 | 66·66 |
| Diatoms .. | 20·00 | 5·88 | .. |
| Cypris .. | .. | 5·88 | .. |
| Crustacean remains .. | .. | 41·17 | 33·33 |

It thus appears that the food of young forms of *K. coval* consists mainly of copepods and other crustaceans. A small percentage of diatoms was also noted in the first two stages.

The stomach of this fish is very muscular forming a gizzard-like structure as is common in *Mugil*. The lumen of the stomach is also small.

*Ilisha brachysoma* (Blkr.)

Larval and post-larval forms of *I. brachysoma* were obtained in small numbers both in plankton and fishermen's catch during the summer and rainy seasons. This fish attains a size of 6–7 inches and is esteemed as food. In all 44 specimens (22–95 mm.), 26 collected in summer and 18 in the rainy season were studied for their food habits during the course of this investigation and the results are stated as under:—

*Number of fish examined and percentage of food taken by each stage*

| Stage of fish | I | II | III | IV |
|---|---|---|---|---|
| Total No. of fish examined .. | 6 | 5 | 20 | 13 |
| No. of fish with digested or no food | 5 | 2 | 4 | 4 |
| Items of food taken : | % | % | % | % |
| Prawn larvæ .. | .. | 66·66 | 50·00 | 33·33 |
| Amphipods .. | .. | .. | 12·50 | 22·22 |
| Fish remains .. | .. | .. | 6·25 | 22·22 |
| Crustacean remains .. | 100 | .. | 31·25 | 22·22 |
| Copepods .. | .. | 33·33 | .. | .. |

An analysis of the percentages of food calculated for each stage shows that the main food of *I. brachysoma* consists of prawn larvæ and is supplemented by copepods in the earlier stages and amphipods and fish food in the third and fourth stages. It has been observed by Chacko (1949) that

the older forms ranging from 140–280 mm. feed on plankton, at times browsing on sea-weeds.

*Ilisha motius* (Ham.)

Larval and post-larval forms of *I. motius* were obtained from Mahim during the rainy and dry seasons. This species was, however, not recorded in plankton collections during the period under review.

A total number of 67 specimens, ranging from 19 to 72 mm. in length, were collected, 43 in rainy and 24 in the dry seasons. Their stomach contents are tabulated below:—

*Number of fish examined and percentage of food taken by each stage*

| Stage of fish | I | II | III |
|---|---|---|---|
| Total No. of fish examined .. | 7 | 27 | 33 |
| No. of fish with digested or no food | 3 | 13 | 15 |
| Items of food taken .. | % | % | % |
| Copepods .. | 100 | 50·00 | 66·66 |
| Pawn larvæ .. | .. | 21·42 | 16·66 |
| Polychætes .. | .. | 21·42 | 11·11 |
| *Gammarus* .. | .. | .. | 5·55 |
| Crustacean remains .. | .. | 7·14 | .. |

*I. motius* seems to be a surface plankton feeder in its early life-history as its food mainly consisted of copepods and prawn larvæ. It is supplemented by polychætes and *Gammarus* to some extent.

*Ilisha filigera* (C.V.)

Post-larval forms of *I. filigera* were collected in small numbers from Mahim during the winter, summer and rainy seasons. This is a good table fish attaining a size of about 12 inches.

*Number of fish examined and percentage of food taken by each stage*

| Stage of fish | II | III |
|---|---|---|
| Total No. of fish examined .. | 30 | 23 |
| No. of fish with digested or no food | 22 | 8 |
| Items of food taken : | % | % |
| Copepods .. | 75·00 | 20·00 |
| Prawn larvæ .. | 25·00 | 20·00 |
| Amphipods .. | .. | 13·33 |
| Mysis .. | .. | 13·33 |
| *Gammarus* .. | .. | 13·33 |
| *Megalops cyprinoides* .. | .. | 6·66 |
| Crustacean remains .. | .. | 13·13 |

In all 53 specimens were collected, 7 in winter, 22 in summer and 24 in the rainy seasons. An analysis of the examination of their stomachs is shown in the above table.

The above table showing the percentages of food taken by each stage tends to show that *I. filigera* is a surface plankton feeder taking comparatively a higher percentage of copepods and prawn larvæ than other organisms.

### *Ilisha elongata* (Benn.)

As many as 75 young forms of *I. elongata* were collected at Mahim almost throughout the year except the winter months. The adults of this fish attain a length of about 18 inches and are considered as highly edible fishes.

*Number of fish examined and percentage of food taken by each stage*

| Stage of fish | II | III |
|---|---|---|
| Total No. of fish examined .. | 43 | 32 |
| No. of fish with digested or no food | 21 | 11 |
| Items of food taken : | % | % |
| Copepods .. | 72·72 | 52·38 |
| Prawn larvæ .. | 9·09 | 33·33 |
| *Gammarus* .. | 18·18 | .. |
| Megalopa .. | .. | 9·52 |
| Zœa .. | .. | 4·76 |

*I. elongata* appears to be a plankton feeder as copepods form the staple food of the young fishes. It will also be seen from the above that copepods are supplemented by *Gammarus* and prawn larvæ in the second and third stages respectively.

### *Thrissocles kammalensis* (Blkr.)

Young forms of *T. kammalensis* occurred in large numbers in the plankton samples taken at Apollo Bunder, Versova and Choupati. According to Day (1882) this fish attains a size of 6 inches. In all 167 specimens ranging from 6–28 mm. only were studied during the present investigation. Of these 41 were collected in rainy, 37 in dry, 15 in winter and 74 in the summer seasons.

It will be seen from the following table that the young forms of *T. kammalensis* feed mainly on copepods and diatoms. The latter were taken only in the summer season by the smaller forms.

*Number of fish examined and percentage of food taken by each stage*

| Stage of fish | I | II |
|---|---|---|
| Total No. of fish examined .. | 160 | 7 |
| No. of fish with digested or no food | 134 | 4 |
| Items of food taken : | % | % |
| Copepods | 69·23 | 100·00 |
| Diatoms .. | 30·77 | .. |

Adults as well as bigger post-larval stages are not commonly found in Bombay waters. Hence the occurrence in large numbers of only the larvæ seems to show that the fish may visit the shallow waters for breeding purposes. This requires further confirmation.

### *Thrissocles hamiltonii* (Gray)

Post-larval and immature forms of *T. hamiltonii* occurred in large numbers in the fishermen's nets at Mahim in the summer and rainy seasons, but not in plankton samples. The adults attain a size of 6–8 inches and are esteemed as food fishes.

A total number of 200 fishes ranging from 22–93 mm. in length were examined during the present investigation. Out of these 103 were obtained in the rainy, 5 in dry, 16 in winter and 76 in the summer seasons. The stage by stage analysis of the stomach contents is given below:—

*Number of fish examined and percentage of food taken by each stage*

| Stage of fish | I | II | III | IV |
|---|---|---|---|---|
| Total No. of fish examined .. | 10 | 76 | 103 | 11 |
| No. of fish with digested or no food | 5 | 17 | 24 | 2 |
| Items of food taken : | % | % | % | % |
| Prawn larvæ .. | 20·00 | 44·06 | 34·17 | 55·6 |
| Copepods .. | 60·00 | 20·35 | 21·51 | 11·1 |
| Polychætes .. | .. | 5·08 | 13·92 | 11·1 |
| Amphipods .. | 20·00 | 3·38 | 16·45 | .. |
| *Gammarus* .. | .. | .. | 2·53 | 11·1 |
| Zoea larvæ .. | .. | 6·77 | .. | 11·1 |
| Gastropods .. | .. | 3·38 | 1·26 | .. |
| Mysis .. | .. | 3·38 | .. | .. |
| Insect larvæ .. | .. | .. | 3·79 | .. |
| Fish remains .. | .. | 1·69 | .. | .. |
| Green algæ .. | .. | .. | 1·26 | .. |
| Crustacean remains .. | .. | 11·86 | 5·06 | .. |

The above table giving the percentage of food taken in each stage clearly indicates that prawns and copepods form the main bulk of food of the species, supplemented by polychætes and amphipods. The percentage of copepods decreases and that of prawn larvæ and polychætes increases as the fish grows in size. The slight decrease in the percentage of prawns and increase in copepods in the third stage may be due to the fact that majority of the specimens of this stage occurred in the rainy season, when the plankton was comparatively rich in copepods.

As regards the seasonal variations in the stomach contents it was found that the fishes of the second and third stages fed more on copepods in the rainy season and of the third stage on prawns in the summer season. Polychætes and *Gammarus* were taken mostly during the rainy season by specimens of the third stage.

*Trissocles purava* (Ham.)

54 Specimens of *T. purava*, 48–198 mm. in size were collected from the fishermen's catch and plankton samples during the rainy and summer seasons. The adults of *T. purava* about 12 inches long are hauled by fishermen in good numbers and are esteemed as good food-fishes.

*Number of fish examined and percentage of food taken by each stage*

| Stage of fish | II | III | IV | Immature (110–198 mm.) |
|---|---|---|---|---|
| Total No. of fish examined .. | 1 | 19 | 14 | 20 |
| No. of fish with digested food or no food | .. | 8 | 7 | 7 |
| Items of food taken : | % | % | % | % |
| Prawn larvæ .. | 50·00 | 54·54 | 100·00 | 69·23 |
| Copepods . | .. | 27·27 | .. | .. |
| Fish diet .. | .. | .. | .. | 15·38 |
| *Gammarus* .. | .. | .. | .. | 7·69 |
| Cypris .. | 50·00 | .. | .. | .. |
| Crustacean remains .. | .. | 18·18 | .. | 7·69 |

It is evident from the above food analysis that *T. purava* is a surface plankton feeder, prawns being the main item. Grown-up forms take prawns belonging to *Acetes* sp. and fish food consisting of *Anchoviella commersonii* and *Trypauchen vagina*.

*Thrissocles dussumieri* (C.V.)

*T. dussumieri* is not a common fish in local waters and it occurred in small numbers in plankton and fishermen's catches. In the course of this investigation only 18 specimens ranging from 25–68 mm. were collected, 3 in rainy, 11 in winter and 4 in the summer seasons.

*Number of fish examined and percentage of food taken by each stage*

| Stage of fish | I | II | III |
|---|---|---|---|
| Total No. of fish examined .. | 2 | 12 | 4 |
| No. of fish with digested or no food | 1 | 4 | .. |
| Items of food taken : | % | % | % |
| Diatoms . | 100·00 | .. | .. |
| Prawn larvæ .. | .. | 75·00 | 50·00 |
| Copepods .. | .. | 12·50 | .. |
| Cypris .. | .. | 12·50 | 50·00 |

*T. dussumieri* seems to feed on planktonic organisms as stated above. Chacko (1949) observes that fish, 90–180 mm. in size, feed on zooplankton.

*Anchoviella commersonii* (Lac.)

Post-larval forms of *A. commersonii*, popularly known as "White-bait" were found in larger numbers at Sassoon Docks, Mahim and Versova, throughout the year with abundance in the rainy and dry seasons. A few specimens were also taken in plankton samples from Apollo Bunder and Versova. Specimens, measuring 2–3 inches, are generally brought by fishermen in large quantities and are consumed as food by the poorer classes of people. The adults attain a size of 8 inches according to Day (1882) and 5 inches according to Weber and Beaufort (1931).

In all 350 specimens, 9–75 mm. in length, were examined, 107 of them in rainy, 104 in dry, 92 in winter and 47 in the summer seasons. An analysis of their food habits according to stages is given below:—

*Number of fish examined and percentage of food taken by each stage*

| Stage of fish | I | II | III |
|---|---|---|---|
| Total No. of fish examined .. | 5 | 195 | 150 |
| No. of fish with digested or no food | 4 | 95 | 84 |
| Items of food taken : | % | % | % |
| Copepods .. | 100·00 | 49·00 | 37·87 |
| Prawn larvæ .. | .. | 10·00 | 37·87 |
| Gastropods .. | .. | 12·00 | .. |
| Diatoms .. | .. | 9·00 | .. |
| Zœa .. | .. | 6·00 | 3·03 |
| Amphipods .. | .. | .. | 6·06 |
| Bivalves .. | .. | .. | 6·06 |
| Polychætes .. | .. | 1·00 | 1·51 |
| Cypris .. | .. | 1·00 | 1·51 |
| Megalopa .. | .. | 2·00 | .. |
| Gammarus .. | .. | .. | 1·51 |
| Mysis .. | .. | 1·00 | .. |
| Crustacean remains .. | .. | 9·00 | 4·54 |

It is evident from the above table that *A. commersonii* is a carnivorous surface plankton feeder, the main food being copepods and prawn larvæ. The percentage of copepods goes on decreasing and that of prawns increasing as the fish grows in size. It has also been observed that the fish fed on a maximum percentage of copepods during the rainy and dry seasons in the second and third stages respectively. Gopinath (1942) also observes that the food of post-larval forms of *A. commersonii* and *A. tri* consists mainly of copepods, lucifers and mysids.

### *Anchoviella tri* (Blkr.)

Only 18 specimens ranging from 33–60 mm. were collected from Mahim creeks on 30th July 1945. According to Day, this fish attains a size of 4 inches, but specimens of this length were not seen in the fishermen's catches or in the local markets.

*Number of fish examined and percentage of food taken by each stage*

| Stage of fish | II | III |
|---|---|---|
| Total No. of fish examined .. | 15 | 3 |
| No. of fish with digested or no food | 6 | .. |
| Items of food taken : | % | % |
| Copepods .. | 88·88 | .. |
| Prawn larvæ .. | 11·11 | 66·66 |
| Zœa .. | .. | 33·33 |

*A. tri* can also be grouped among the plankton feeders like some other members of its family.

### *Coilia dussumieri* (C.V.)

Post-larvæ and adults of this species occur in large numbers practically throughout the year in Bombay and are abundant during the rainy season,

*Number of fish examined and percentage of food taken by each stage*

| Stage of fish | II | III | IV | Adults (110–180 mm.) |
|---|---|---|---|---|
| Total No. of fish examined .. | 31 | 65 | 104 | 27 |
| No. of fish with digested or no food | 13 | 26 | 50 | 11 |
| Items of food taken : | % | % | % | % |
| Copepads .. | 44·44 | 46·15 | 29·62 | 18·75 |
| Prawn larvæ .. | 11·11 | 30·76 | 42·59 | 31·25 |
| Cypris .. | 38·88 | 15·38 | 16·68 | 37·50 |
| Zœa .. | .. | .. | 3·70 | .. |
| Crustacean remains .. | 5·55 | 7·69 | 7·40 | 12·50 |

but the larval forms were not found either in plankton or fishermen's catches during the course of this study. The adults are locally known as " Mandeli " and are esteemed as delicious food. As many as 227 specimens, ranging from 28–180 mm. were obtained from Sassoon Docks, Versova, Danda and Mahim creeks. 111 of them were collected in the rainy, 15 in dry, 50 in winter and 51 in the summer seasons. An examination of their stomach contents, stage by stage, is given in the previous table.

It appears from the above table that *C. dussumieri* is a selective plankton feeder as it takes mainly copepods, prawn larvæ and cypris excluding the other organisms such as amphipods, sagittæ, crustacean larvæ, etc., which were common in plankton. It has also been observed that the occurrence of cypris in plankton is an indication of the appearance of this fish in large numbers in local waters.

This fish is generally found infected with nematodes, trematodes and larvæ of cestodes.

## Conclusions

In the course of this investigation extending over two years, 1945–47, the stomach contents of the following 16 species of Clupeids, namely, *Nematolosa nasus*, *Harengula punctata*, *Sardinella albella*, *Hilsa toli*, *Kowala coval*, *Ilisha brachysoma*, *I. motius*, *I. filigera*, *I. elongata*, *Thrissocles kammalensis*, *T. hamiltonii*, *T. purava*, *T. dussumieri*, *Anchoviella commersonii*, *A. tri*, and *Coilia dussumieri* were examined for the determination of their food habits.

1. Most of the species studied are surface plankton feeders in the early part of their life-history.

2. All the species, except *Hilsa toli*, are carnivorous in their habits in the young stages. *H. toli* can, however, be classified as an omnivorous feeder due to high percentage of datoms also in their stomachs.

3. *Harengula punctata* and *Nematolosa nasus* may be included among the bottom feeders as sand and mud were found in a large quantity in the stomachs examined.

4. The stomachs of *Nematolosa nasus* and *Kowala coval* are more muscular and gizzard-like in appearance as seen in *Mugil.*

5. The larvæ and post-larvæ of most of the above forms generally feed on a large percentage of copepods. The bigger forms take to larger proportions of prawn larvæ than copepods. They further supplement their food with crustacean larvæ, amphipods and *Gammarus* in smaller proportions.

6. Molluscan larvæ (gastropods and bivalves) were taken in smaller percentages by *Anchoviella commersonii* alone.

7. *Thrissocles hamiltonii* is the only species which seems to feed on polychætes as well, judging from the appreciable quantity of worms in all but the first stage, although its main food consists of prawns and copepods.

8. The occurrence of larvæ and post-larvæ of Clupeids in the summer and early rainy seasons only seems to indicate that the majority of them breed during the period covered by the two seasons.

## BIBLIOGRAPHY


Allen, K. Radway .. "Studies on the Biology of the early stages of the Salmon," *J. Anim. Ecol.*, 1940, **9**, 1–23.

Bal, D. V. and Pradhan, L. B. .. "A preliminary note on the plankton of Bombay Waters," *Curr. Sci.*, 1945, **14**, 211–12.

Bullen, C. E. .. "Plankton studies in relation to the Western Mackerel Fishery," *J. Mar. Bio. Ass. U.K.*, 1908, **8**, 269–302.

———— .. "Some notes on the feeding habits of Mackerel and certain Clupeids in the English Channel," *ibid.*, 1910-13, **9**, 394–403.

Chacko, P. I. .. "On the bionomics of the big jawed jumper *Lactarius lactarius*," *Curr. Sci.*, 1944, **13**, 108.

———— .. "Bionomics of the Leiognathidæ," *ibid.*, 1944, **13**, 214.

———— .. "On the food and the alimentary canal of the milk-fish, *Chanos chanos*," *ibid.*, 1945, **14**, 242.

———— .. "On the bionomics of the silver fish *Chela argentia* (Day)," *ibid.*, 1946, **15**, 167.

———— .. "Food and feeding habits of the fishes of the Gulf of Manaar," *Proc. Ind. Acad. Sci.*, Sec. B, 1949, **29**, 83.

—— and Venkatraman, R. S. .. "On the food of mullets," *Curr. Sci.*, 1945, **14**, 79.

Chadwick, H. C. .. "Feeding habits of the angler-fish *Lophius piscatorius*," *Nature*, 1929, **124**, 337.

Chidambaram, K. .. "Food of the Indian Mackerel (*Rastrelliger kanagurta* Russel.) of the West Coast of Madras Presidency," *Curr. Sci.*, 1944, **13**, 214–15.

Day, F. .. "The food of Herring," *Zoologist*, 1882, **3**, Ser. 6, 268.

———— .. "On the food of the sea fishes," *ibid.*, 1882, **3**, Ser. 6, 235.

———— .. "The food of fishes," *Fish-Exhib. Liter*, Lond., 1883, VI Confer., Pt. 3, 265–300.

———— .. *Fauna of British India, Fishes*, 1889, Vols. I and II.

Devanesen, D. W. .. "Plankton studies in the fisheries branch of the Department of Industries and Commerce, Madras," *Curr. Sci.*, 1942, **11**, 142.

—— and Chacko, P. I. .. "Balanoglossus as food of fishes," *ibid.*, 1942, **11**, 242.

Forbes, S. A. .. "The food of the young white fish (*Corregonnus clupeiformes*)," *Bull. U.S. Fish. Comm.*, **1**, 19, 269 and 403.

Gonzalves, Ella. A. .. "Variations in the seasonal composition of the Phytoplankton of Bombay Harbour," *Curr. Sci.*, 1947, **16**, 304.

George, C. J. and Desai, N. S. .. "The Biology of the Fishes of the Mahim creek," *J. Univ. Bombay*, 1944, **12**, 46–54.

Gopinath, K. .. "Distribution and feeding of the post-larval fishes of the Trivandrum coast," *Curr. Sci.*, 1942, **11**, 337.

———— .. "Notes on the larval and post-larval stages of fishes found along the Trivandrum coast," *Proc. Nat. Inst. Sci. India*, 1946, **12**, No. 1, 7–21.

Hardy, A. C. .. "Herring in relation to its animate environment, Pt. I. The food and feeding habits of the herring with special reference to the East coast of England," *Min. Agric. and Fish, Fisheries Investigation*, 1924, Ser. II, **7**, No. 3.

Herdman, W. A. .. "Report on the Investigation carried on during 1916, in connection with the Lancashire sea fisheries laboratory," *Proc. & Trans. Liverpool, Biol. Soc.*, 1917, **31**, 77.

—— and Corbin, P. E. I. .. "The food of young fishes," *Rep. Lancashire Sea-fish Lab.*, 1892, p. 10.

—— and Scott, A. .. "Food of fishes," *ibid.*, 1893, 3.

———— .. "The food of young fishes," *ibid.*, 1894, 3.

Hickling, C. F. .. "The seasonal cycle in the Cornish Pilchard, *Sardina Pilchardus* Walbaum," *J. Mar. Bio. Ass. U.K.*, 1945, **26**, 115.

Hora, S. L. .. "Wanderings of the Bombay Duck, *Harpodon nehereus* (Ham. Buch.) in Indian Waters," *J. Bombay Nat. Hist Soc.*, 1934, **37**, 610–54.

———— .. "Bionomics of Hilsa (Food of young Hilsa)," *Rec. Indian Mus.*, 1938, **40**, 155.

—— and Nair, K. K. .. "Observation on the nutrition of *Panchax panchax* (Ham.)," *Proc. Nat. Inst. Sci. India*, 1938, **4**, 245–51.

Hornell, J. and Naidu Ramaswamy, M. "A contribution to the life-history of the Indian Sardine with notes on the Plankton on the Malabar coast," *Madras Fish. Bull.*, 1923, **17**, 129–97.

Job, T. J. .. "Nutrition of Madras Perches," *Rec. Indian Mus.*, 1940, **42**, 286–364.

John, C. C. and Menon, M. A. S. "Food and feeding habits of the Oil-Sardine and Mackerel," *Curr. Sci.*, 1942, **11**, 243–44.

Johnstone, J. .. "The food fishes," *Rep. Lancashire Sea-Fish Lab.*, 1906, 216.

———— .. "The food of fishes," *Trans. Liverpool Biol. Soc.*, 1907, **21**, 316–27.

Johnstone, Scott, A. and Chadwick *The Marine Plankton*, Liverpool University Press, 1924.

Kulkarni, C. V. .. "On the systematic position, structural modifications, bionomics and development of a remarkable new family of Cyprinodont fishes from the Province of Bombay," *Rec. Indian Mus.*, 1940, **42**, 379–423.

Lebour, M. V. .. "Food of post-larval fish," *J. Mar. Bio. Ass. U.K.*, 1918, **2**, 433–69.

———— .. "The food of post-larval fish," *ibid.*, 1919, **12**, 22–47.

———— .. "Feeding habits of some young fish," *ibid.*, 1919, **12**, 9-21.

———— .. "The food of young fishes," *ibid.*, 1919, **12**, 261–324.

———— .. "The food of young Clupeoids," *ibid.*, 1921, **12**, 458–67.

———— .. "The food of young Herrings," *ibid.*, 1924, **13**, 325-30.

———— .. "Rissoid larvæ as food of young Herring. The eggs and larvæ of the Plymouth Rissoidæ," *ibid.*, 1934, **19**, 523–40.

Marshall, S. M., Nichols, A. G. and Orr, A. P. "On the growth and feeding of young Herring in the Clyde," *ibid.*, 1929, **23**, 427–55.

Menon, M. A. S. .. "Observations on the seasonal distribution of the plankton of the Trivandrum coast," *Proc. Ind. Acad. Sci.*, 1945, **22**, Sec. B, 31–62.

Mookerjee, H. K. .. "Food and its percentage composition of the common adult food fishes of Bengal," *Science and Culture*, 1946–47, **12**, 247.

Norman, J. R. .. *A History of Fishes*, London, 1936.

Ogilvie, H. S. .. "Observations on the food of Post larval Herring from the Scottish coast," *Fish Scotland Sci. Invest.* 1927. **1**.

Pearse, A. S. .. "The food of the shore fishes of certain Wisconian lakes," *Bull. U. S. Bur. Fish.*, 1916, **35**, 245–92.

———— .. "The food of the shore fishes in the waters near Madison, Wisconian," *Bull. Wisc. Nat. Hist. Soc.*, 1916, **13**.

Russell, F. S. and Yonge, C. M. *The Seas*, London and New York, 1936.

Scott, Andrew .. "The food of young fishes," *Rep. Lanc. Sea-fish. Lab.*, 1895, 6.

———— .. "Observations on the habits and food of young fishes," *Lanc. Sea-fish. Report*, 1898, 22.

———— .. "Notes on the food of young fishes," *Rep. Lanc. Sea-fish. Lab.*, 1906, 99.

———— .. "On the food of young Plaice," *J. Mar. Bio. Ass. U.K.*, 1922, **12**, 678–87.

Scott, T. .. "Observations on the food of fishes," *20th Ann. Rep. Fish. Bd. Scotland*, 1902, Pt. III, 486–538.

———— .. "Some observations on the food of Herring," *25th Ann. Rep. Fish. Bd. Scotland*, 1906, Pt. III, 260–71.

———— .. "Examination of food in fish stomachs," *Proc. Trans. Liv. Biol. Soc.*, 1898, 108–14.

Sewell, R. B. S. .. "The Copepoda of Indian Seas," *Mem. Ind. Mus.*, 1929, **10**.

Sim, G. .. "The food of fishes," *Fish. Exhib. Liter.*, London, Prize Essays, Pt. IV, 1883. XI, 499–508.

Sorley, H. T. .. *The Marine Fisheries of the Bombay Presidency*, Govt. Central Press, Bombay, 1937.

Steven, G. A. .. "The food consumed by Shags and Cormorants around the shores of Cornwall (England)," *J. Mar. Bio. Ass. U.K.*, 1933, **19**, 277–92.

——— .. "Bottom fauna and the food of fishes," *ibid.*, 1930, **16**, 677–706.

Swynnerton, G. H. and Worthington, E. B. "Notes on the food of fish in Hawes-Water (Westmorland)," *J. Anim. Ecol.*, 1940, **9**, 183–87.

Todd, R. A. .. "Notes on the Invertebrate Fauna and Fish Food of the Bays between the Start and Exmouth," *J. Mar. Bio. Ass.*, 1903, **6**, 541–61.

Venkatraman, R. S. .. "The food of the ribbon fish *Trichiurus* sp.," *Curr. Sci.*, 1944, **13**, 239.

Weber and Beaufort .. *The Fishes of the Indo-Australian Archipelago*, Vols. II to VIII, 1913–40.

320-50 Printed at The Bangalore Press, Mysore Road, Bangalore City, by G. Srinivasa Rao, Superintendent, and Published by The Indian Academy of Sciences, Bangalore

## ERRATUM

*Vol. XXXI, No. 5, Sec. B, May 1950, Page 255*

" The histogram relating to 1942–43 in Fig. 1 is to be shifted to the right by 5 cms."

# PHYSICO-CHEMICAL STUDIES ON PAPAYA AND INDIAN GOOSEBERRY PECTINS

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It is now generally accepted that the pectinic acid complex extracted from most plant sources consists of a long chain skeleton of partially methyl-esterified polygalacturonic acid to which are attached side groups of araban and galactan (Bauér and Link, 1935; Bevan and Jones, 1939; Hirst and Jones, 1939; Morrel, Bauér and Link, 1934 *a*; Morrel, Bauér and Link, 1934 *b*; Morrel and Link, 1934; Schneider and Bock, 1937; Schneider and Fritschi, 1936; Smith, 1939). Pectinic acid is not homogeneous, for the methyl ester, araban and galactan content of a sample as well as the molecular weight vary with the source, methods of extraction and subsequent treatment (Norman, 1937; Norris and Resch, 1937; Speiser, Eddy and Hills, 1945; Hills and Speiser, 1946; Speiser and Eddy, 1946). It has been shown by Schneider and Bock (1938) that the characteristic properties of pectin, *viz.*, gelation, film formation, acid behaviour and high viscosity in dilute solutions are principally properties of the polygalacturonide chain, the non-galacturonide constituents acting mainly as diluents. More information on the various physico-chemical properties of pectin preparations are desired to assist in its evaluation for new uses.

The distribution, isolation and purification of high grade pectins from a variety of indigenous plant materials have been dealt with in a previous paper (Krishnamurti and Giri, 1949). Of these materials, papaya and Indian gooseberry were found to be very promising as rich sources of pectin and in view of the popularity of these as edible fruits, a detailed investigation of the pectin complex isolated from them was thought of interest. Certain physico-chemical properties and jelly formation under different conditions of papaya and gooseberry pectins will form the subject of the present communication.

## Experimental

The pectins prepared by ammonium oxalate extraction were used in the following studies. The pectins were purified by dialysis, bromination and repeated precipitation with alcohol. The composition of the pectins thus obtained is given in Table I.

TABLE I

*Composition of purified papaya and gooseberry pectins*

| | Ash % | Calcium pectate % | Uronic anhydride % | Methoxyl value % |
|---|---|---|---|---|
| Papaya .. | 0·27 | 74·28 | 66·34 | 7·08 |
| Indian gooseberry .. | 0·21 | 43·82 | 32·37 | 4·92 |

The methods used for the determination of the above figures have been described elsewhere (Krishnamurti and Giri, *loc. cit.*).

*Preparation of metallic compounds.*—Copper, lead, barium and strontium compounds of the two pectins were prepared according to the method described by Ghose, Krishna and Rao (1946). The results obtained are presented in Table II.

TABLE II

*Metal content of metal pectin complexes*

| | Copper pectin Cu % | Lead pectin Pb % | Strontium pectin Sr % | Barium pectin Ba % |
|---|---|---|---|---|
| Papaya .. | 7·25 | 18·31 | 7·62 | 9·32 |
| Indian gooseberry .. | .. | 23·46 | 7·56 | 8·75 |

For the recovery of pectins from metal pectin complexes, the latter were dispersed in water and treated with warm HCl, heated on a water-bath for 15 minutes, cooled and filtered. The filtrate on precipitation with three times the volume of alcohol yielded the pectin. Recovery of pectins from the complexes ranged from 68 to 81 per cent. Jellying power was not lost during the reaction. There was no material improvement, however, as regards the purity of the pectins by the above treatment.

*Acid hydrolysis of pectins.*—The hydrolysis of the pectins was accomplished in 2·5% $H_2SO_4$ by refluxing for 15 hours. In the initial stages, charring was carefully avoided by regulating the flame. After hydrolysis was over, the excess of sulphuric acid was neutralised by the addition of baryta and $BaCO_3$ in suitable proportions. The precipitated $BaSO_4$ was removed by filtration under suction and the resulting filtrate was concentrated under vacuum. Barium galacturonate was recovered from the concentrate by precipitation with alcohol. Yields of barium galacturonates from papaya and Indian gooseberry were 16·84 and 12·21 respectively. Barium contents of the salts were 27·18

and 27·61 respectively. The barium galacturonates were converted into galacturonic acid by hydrolysis with 0·02 N $H_2SO_4$ and was finally identified by preparing the brucine salt (Ehrlisch and Schubert, 1929).

TABLE III

*Effect of concentration of pectin on relative viscosity at 25° C.*

| | Concentration as percentage | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0·005 | 0·01 | 0·025 | 0·05 | 0·10 | 0·30 | 0·50 | 0·80 | 1·0 |
| Papaya .. | 1·044 | 1·092 | 1·123 | 1·238 | 1·443 | 1·527 | 1·738 | 2·031 | 2·327 |
| Indian gooseberry .. | 1·003 | 1·013 | 1·092 | 1·178 | 1·213 | 1·412 | 1·617 | 1·819 | 1·932 |

TABLE IV

*Effect of temperature on relative viscosity*

A : Papaya pectin B : Indian gooseberry pectin

| Concentration as per cent. | Temperature Degrees Centigrade | | | | | | |
|---|---|---|---|---|---|---|---|
| | 15° | 25° | 30° | 35° | 40° | 45° | 50° |
| 0·005 | A 1·044 | 1·044 | 1·044 | 1·044 | 1·044 | 1·044 | 1·044 |
| | B 1·003 | 1·003 | 1·003 | 1·003 | 1·003 | 1·003 | 1·003 |
| 0·010 | A 1·092 | 1·092 | 1·092 | 1·092 | 1·092 | 1·092 | 1·092 |
| | B 1·013 | 1·013 | 1·013 | 1·013 | 1·013 | 1·013 | 1·013 |
| 0·025 | A 1·123 | 1·123 | 1·123 | 1·123 | 1·123 | 1·123 | 1·123 |
| | B 1·092 | 1·092 | 1·092 | 1·092 | 1·092 | 1·092 | 1·092 |
| 0·050 | A 1·238 | 1·238 | 1·238 | 1·238 | 1·238 | 1·238 | 1·238 |
| | B 1·178 | 1·178 | 1·178 | 1·178 | 1·178 | 1·178 | 1·178 |
| 0·100 | A 1·443 | 1·443 | 1·443 | 1·443 | 1·443 | 1·443 | 1·443 |
| | B 1·213 | 1·213 | 1·213 | 1·213 | 1·213 | 1·213 | 1·213 |
| 0·300 | A 1·527 | 1·527 | 1·527 | 1·527 | 1·527 | 1·520 | 1·516 |
| | B 1·412 | 1·412 | 1·412 | 1·412 | 1·412 | 1·409 | 1·403 |
| 0·500 | A 1·738 | 1·736 | 1·731 | 1·715 | 1·619 | 1·680 | 1·671 |
| | B 1·617 | 1·617 | 1·613 | 1·608 | 1·602 | 1·598 | 1·587 |
| 0·800 | A 2·031 | 2·020 | 2·009 | 1·991 | 1·971 | 1·934 | 1·872 |
| | B 1·819 | 1·814 | 1·800 | 1·800 | 1·791 | 1·753 | 1·717 |
| 1·000 | A 2·327 | 2·308 | 2·264 | 2·203 | 2·181 | 2·113 | 2·093 |
| | B 1·932 | 1·921 | 1·907 | 1·883 | 1·843 | 1·821 | 1·807 |

TABLE V

*Effect of sugar concentration on jelly strength*

Different amounts of sugar added to pection solution containing 0·08 gm. of pectin as pectate and 0·24 gm. of citric acid.

A : Papaya pectin B : Indian gooseberry pectin

| Sugar added in gm. | Yield of jelly in gm. | Jelly strength in seconds | Sineresis temperature °C. | % Acid in jelly | % Sugar in jelly | % Pectin in jelly |
|---|---|---|---|---|---|---|
| 10·0 | A 16·3 | 116 | 62 | 1·44 | 60·5 | 0·420 |
| | B 15·8 | 112 | 58 | 1·48 | 61·0 | 0·390 |
| 12·5 | A 20·5 | 84 | 61 | 1·11 | 61·0 | 0·340 |
| | B 20·1 | 92 | 53 | 1·21 | 61·8 | 0·360 |
| 15·0 | A 24·0 | 75 | 60 | 1·01 | 63·0 | 0·290 |
| | B 24·0 | 90 | 43 | 1·03 | 62·5 | 0·280 |
| 20·0 | A 31·2 | 70 | 51 | 0·72 | 63·5 | 0·220 |
| | B 32·0 | 81 | 40 | 0·79 | 63·0 | 0·270 |
| 30·0 | A 45·0 | 22 | 40 | 0·51 | 64·0 | 0·150 |
| | B .. | .. | .. | .. | .. | .. |
| 40·0 | A 60·0 | 12 | 32 | 0·36 | 65·0 | 0·110 |
| | B 62·0 | 17 | 30 | 0·39 | 64·7 | 0·130 |
| 50·0 | A 72·8 | 9 | Room Temp. | 0·31 | 66·8 | 0·090 |
| | B 75·0 | 11 | ,, | 0·29 | 27·2 | 0·090 |

The sugar units liberated during hydrolysis were identified in the alcoholic filtrate after separation of the barium salt of galacturonic acid. From the mixed phenyl osazones presence of arabinose and galactose were confirmed. The presence of galactose in the pectins was further confirmed by preparation of mucic acid. The yields of mucic acid from papaya and gooseberry pectins were 8·27 and 6·14 respectively.

The general procedure followed for hydrolysis and recovery of the products was according to Bridgeham and King (1928).

*Viscosity of pectin solutions.*—Different concentrations of pectin solutions were made and the time of flow for a fixed volume of solution through a particular length of the capillary in an Ostwald viscosimeter were measured under constant temperature in a thermostat (25° C). Densities of the solutions were determined by the specific gravity bottle method and the relative viscosities were determined from the corresponding time of flow for distilled water. Viscosities were further determined at different temperatures. The results obtained are embodied in Tables III and IV.

TABLE VI

*Effect of acid on pectin jellies*

Sugar concentration: 60 gm. and solution of pectin containing 0·38 gm. boiled with different amounts of acid.

A: Papaya pectin B: Indian gooseberry pectin

| Normal solution added in c.c. | Citric acid | Tartaric acid | Sulphuric acid |
|---|---|---|---|
| | Jelly strength in seconds :— | | |
| 0·50 | A 58<br>B no gel. | 51<br>no gel. | 78<br>67 |
| 1·00 | A 65<br>B no gel. | 70<br>no gel. | 70<br>62 |
| 2·00 | A 70<br>B no gel. | 76<br>no gel. | 63<br>59 |
| 4·00 | A 75<br>B no gel. | 84<br>no gel. | 46<br>51 |
| 5·00 | A 80<br>B no gel. | 82<br>no gel. | 27<br>49 |
| 6·00 | A 69<br>B no gel. | 68<br>42 | 18<br>28 |
| 7·00 | A 52<br>B 27 | 50<br>49 | no gel.<br>12 |
| 8·00 | A 41<br>B 39 | 38<br>57 | no gel.<br>no gel. |
| 9·00 | A 40<br>B 70 | 33<br>72 | no gel.<br>no gel. |
| 10·00 | A 38<br>B 58 | 32<br>63 | no gel.<br>no gel. |

When the concentration was plotted against the relative viscosity, a curve similar to those for ionisable hydrophylic colloids was obtained.

It is thus clear that up to 0·50% there is no visible change in the relative viscosity with increase in temperature.

*Pectin-sugar-acid jellies under different conditions.*—Jellies were prepared according to the procedure adopted by Olsen, *et al.* (1939) and the jelly strength was determined by the method suggested by Singh and Dutt (1941). The results obtained are summarised in Tables V, VI and VII.

TABLE VII

*Effect of pectin concentration on jelly strength*

60 gm. of sugar and 0·24 gm. of citric acid.

A : Papaya pectin B : Indian gooseberry pectin

| Pectin added gm. | Jelly strength in seconds | Pectin content of jelly % | Sugar in jelly % |
|---|---|---|---|
| 0·08 | A 81 | 0·21 | 65·8 |
| | B 73 | 0·19 | 64·8 |
| 0·23 | A 83 | 0·51 | 62·3 |
| | B 76 | 0·31 | 63·2 |
| 0·39 | A 91 | 0·89 | 59·1 |
| | B 81 | 0·47 | 61·7 |
| 0·56 | A 103 | 1·19 | 56·4 |
| | B 92 | 0·78 | 60·4 |
| 0·72 | A 112 | 1·56 | 53·8 |
| | B 98 | 0·89 | 59·0 |
| 0·87 | A 109 | 1·98 | 50·7 |
| | B 90 | 1·67 | 56·0 |
| 1·21 | A 98 | 2·31 | 48·9 |
| | B 87 | 1·98 | 49·3 |

## DISCUSSION

Pectins are characterised by the presence of methyl ester groups and galacturonic acid units with varying amounts of arabinose and galactose attached to the molecule. Two pectin preparations obtained from two entirely different sources have yielded the same products on acid degradation, *viz.*, polygalacturonic acid, arabinose and galactose. One characteristic feature of the two pectins studied is their comparatively low methoxyl value. 11 to 13 % of methoxyl as total esterified groups is reported for citrus and apple pectins (Norman, 1937). Yet, the pectins have yielded good jellies. This would lead one to doubt the hypothesis relating jelly strength of pectin to its methoxyl value.

The viscosity of the pectin solution is similar to that of long chain solvated polymers like gum arabic (Kruyt, *et al.*, 1931), agar (Kruyt, 1928) and polyacrylic acid (Kruyt, *et al.*, 1938). Calculation of molecular weight of pectins from the viscosity data has also given values which approach those of long chain polymers,

It would be clear from the results obtained by studying the influence of different factors on jelly strength that increasing the concentration of sugar not only reduces the jelly strength but prevents jelly formation beyond a particular concentration. 60 to 61 % of sugar in the jelly seems to be ideal for both the pectins studied. When the concentration of pectin is increased there is, however, a corresponding decrease in the amount of sugar needed for good jellying. The optimum concentrations of sugar, acid and pectin for good jelly formation seem to be 60%, 1·2 to 1·1 % as lactic acid and 0·5 to 0·8 % pectin as calcium pectate respectively.

## Summary

1. Highly purified pectin preparation from papaya and Indian gooseberry have been shown to form typical metal complexes and yield polygalacturonic acid, arabinose and galactose on acid hydrolysis.

2. The viscosities of the pectin solutions were of the order usually found for long chain solvated polymers. Up to 0·5 % concentration of pectin there was no visible change in relative viscosity with increase in temperature.

3. The formation of jellies under different concentrations of sugar, acid and pectin has been studied.

## Acknowledgements

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## References

Bauér, L. and Link, K. P. .. *J. Biol. Chem.*, 1935, **109**, 293.
Beven, G. H. and Jones, J. K. N. .. *Chem. & Ind.*, 1939, **17**, 363.
Bridgeham, L. N. and King, C. G. .. *J. Am. Chem. Soc.*, 1928, **51**, 3119.
Ehrlisch, P. and Schubert, H. .. *Ber. Chem. Ges.*, 1928, **62**, 1674.
Ghose, T. P., Krishna, S. and Rao, P. S. .. *J. Sc. & Ind. Res.*, 1946, **4**, 705.
Hirst, E. L. and Jones, J. K. N. .. *J. Chem. Soc.*, 1939, 454.
Hills, C. H. and Speiser, R. .. *Science*, 1946, **103**, 166.
Krishnamurti, C. R. and Giri, K. V. .. *Proc. Ind. Acad. Sci.*, B, 1949 **29**, 155.
Kruyt, H. R. and de Jong, H. B. .. *Kolloidchimie, Beihefte*, 1928, **28**, 1.
—— and Winkler, K. C. .. *Ibid.*, 1931, **32**, 374.
Morrel, S., Bauér, L. and Link, K. P. .. *J. Biol. Chem.*, 1934, **105**, 1.
———— .. *Ibid.*, 1934, **105**, 15.
—— and Link, K. P. .. *Ibid.*, 1934, **104**, 183.

Norman, A. G. .. *Biochemistry of Cellulose Polyuroniles and Lignin*, London, 1937.

Norris, F. W. and Resch, C. E. .. *Biochem. J.*, 1937, **31**, 1945.

Olsen, A. G., Steuver, R. F., Fehlbery, E. R. and Beach, N. M. *Ind. Eng. Chem.*, 1939, **31**, 1015.

Owens, H. S., Lotzker, H., Merril, R. and Peterson, M. *Jour. Am. Chem. Soc.*, 1944, **66**, 1178.

Schneider, G. G. and Bock, H. .. *Ber.*, 1937, **B 70**, 1617.

——— and Fritschi, O. .. *Ibid.*, 1936, **B 69**, 2537.

———, ——— and Bock, H. .. *Ibid.*, 1938, **B 71**, 1353.

Smith, F. .. *Chemistry and Industry*, 1939, **17**, 363.

Speiser, R., Eddy, C. R. and Hills, C. H. .. *Jour. Physical Chem.*, 1950, **49**, 583.

Speiser, R. and Eddy, C. R. .. *Jour. Am. Chem. Soc.*, 1946, **68**, 287.

Singh, B. N. and Dutt, B. .. *Ind. Jour. Agr. Sci.*, 1941, **11**, 1006.

# ADDITIONS TO FUNGI OF MADRAS—VII

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Received May 2, 1949

(51) *Synchytrium crotalariæ* Ramakrishnan, T. S. and K., sp. nov.

Galls single or in groups, on leaves and stem, bright orange when young, cupulate after dehiscence, 1–2 mm. diam., amphigenous, more on the lower surface; resting spores not seen; summer sporangia forming large sori up to 500 $\mu$ in diam.; sporangia 26 × 24 $\mu$ (20–34 × 17–34), globose or angular, orange coloured, smooth, thin-walled.

Gallæ in foliis et culmis, singulæ vel aggregatæ, aurantiacei colores in juventute, cupulatæ, 1–2 mm. diam.; hypnosporæ ignotæ; soris usque 500 $\mu$ diam.; sporangiis numerosis, globosis vel angulatis, levis, 26 × 24 $\mu$ (20–34 × 17–34); contentu aurantiaco.

On living leaves and stems of *Crotalaria semperflorens* Vent. (Papilionaceæ), Lovedale, Ootacamund, 17-1-1949, T. S. Ramakrishnan.,

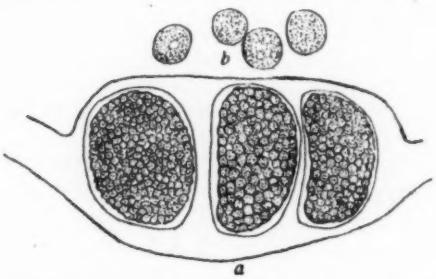


Fig. 1. *Synchytrium crotalariæ*, (*a*) section of leaf with sori (diagrammatic), (*b*) sporangia × 250.

Numerous galls are seen on the leaves and young stems. When young they are bright orange coloured. On dehiscence they become cupulate. In each gall 2–5 chambers are visible. The sori are made up of numerous sporangia. Resting spores were not observed.

(52) *Pseudopeziza indica* Ramakrishnan, T. S. and K., sp. nov.

Spots amphigenous, brown; apothecia hypophyllous, innate, later erumpent, irregularly circular, sessile; asci clavate, hyaline, paraphysate,

paraphyses filiform, not branched, 85 × 12 μ (72–112 × 8–16); ascospores 8, globose, hyaline, one-cellled, 8·4 × 8 μ (6–12 × 6–12).

Maculæ amphigenæ, brunneæ; apothecia hypophylla, innata, dien erumpentia, irregulariter circularia, sessilia; asci clavati, hyalini 85 × 12 μ (72–112 × 8–16), paraphysati, paraphyses filiformes, simplices; ascosporidia 8, globosa, hyalina, unicellata, 8·4 × 8 μ (6–12 × 6–12).

On living leaves of *Embelia ribes* Burm. (Myrsinæ), Anamalais, 30–1–1949, T. S. Ramakrishnan.

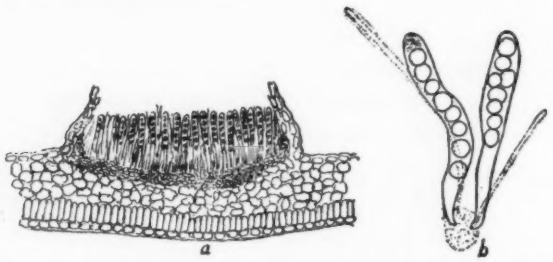


FIG. 2. *Pseudopeziza indica*, (*a*) section through apothecium (semidiagrammatic), (*b*) asci and paraphyses × 270.

The apothecia open out on the lower surface of the leaf. The edges of the apothecia are lacerated and incurved. Fresh apothecia are translucent and whitish but on drying they become black.

(53) *Physalospora anamalaiensis* Ramakrishnan, T. S. and K., sp. nov.

Spots epiphyllous, indefinite, slightly raised, reddish; perithecia punctiform, black, innate, crowded in the spot, globose, ostiolate, rather deep-seated, but not reaching up to the lower epidermis, wall composed of two or three layers of thin-walled cells; asci hyaline, fusiform or cylindrical with a small foot, paraphysate, paraphyses filiform, wall gelatinising in water, 110 × 12 μ (82–129 × 10–17): ascospores 8, hyaline, elliptical to oblong, one-celled, wall gelatinising in water, 13 × 10 μ (10–17 × 7–14).

Maculæ epiphyllæ, indefinitæ, rubrei colores; perithecia punctiformia, atra, innata, globosa, profunde immersa; asci hyalini, fusiformi vel cylindrici, brevipedicellati, paraphysati, paraphyses filiformes, murus asci gelatinous in aquæ, 110 × 12 μ (82–129 × 10–17); ascosporidia 8, hyalina, elliptica vel oblonga, unicellata, 13 × 10 μ (10–17 × 7–14).

On living leaves of *Embelia ribes* Burm., Anamalais, 30–1–1949, T. S. Ramakrishnan.

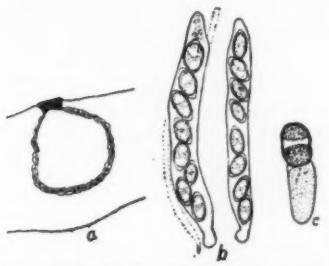


FIG. 3. *Physalospora anamalaiensis*, (*a*) section through perithecium × 45, (*b*) asci and paraphysis × 270, (*c*) Pycnidiospore × 540.

The infection spots are not well defined but appear as convex reddish patches on the upper surface studded with many black dots representing the location of the perithecia. A black clypeus-like development is seen at the mouth of the perithecium. On the lower surface of the leaf brown dots are visible corresponding to the position of the perithecia. The asci are straight or slightly curved. The ascospores exhibit a gelatinous covering when mounted in water.

Associated with this fungus are globose pycnidia in the tissues of the leaf. Inside these pycnidia are formed one-celled, dark brown elliptic pycnidiospores. Each spore has a hyaline equatorial band. The pycnidial locules open eiphyllously. The pycnidiospores measure 9·5 × 7 $\mu$ (7–10 × 5–8·5). They have broad stalks and appear to be formed endogenously. Though they occur together the relationship between these two has not been established.

*Physalospora embeliæ* Yates, *Phyllachora embeliæ* V. Hohn., and *P. secunda* V. Hohn., have been recorded on *Embelia ribes* and other species (Saccardo, 1926, 1928). The fungus under study differs from these in having much bigger asci and ascospores.

(54) *Ascomycetella symploci* Ramakrishnan, T. S., sp. nov.

Spots amphigenous, circular, brown; stromata crowded, isolated or confluent, subepidermal, erumpent, gelatinising; asci elliptical, or oblong, hyaline, inordinate, crowded, 66 × 33 $\mu$ (33–103 × 22–42); ascospores 8, oblong, muriform, many celled, hyaline, 29 × 11 $\mu$ (19–34 × 8–14).

Maculæ amphigenæ, orbiculares, brunneis; stromata gregaria, isolata vel confluenta, subepidermalia, erumpentia, gelatinosa; asci elliptici vel oblongi, hyalini, crebrimi, inordinati, 66 × 33 $\mu$ (33–103 × 22–42); ascosporidia 8, oblonga, muriformia, hyalina, pluricellata, 29 × 11 $\mu$ (19–34 × 8–14).

On living leaves of *Symplocos spicata* Roxb. (Symplocaceæ), Sims Park, Coonoor, 24–3–1948, G. Rangaswamy and S. Damodaran.

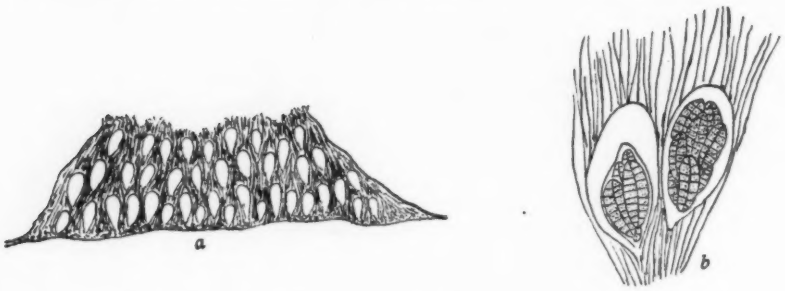


FIG. 4. *Ascomycetella symploci*, (*a*) section through stroma (diagrammatic), (*b*) asci and paraphyses × 300.

The spots are visible on both sides of the leaf. On the upper surface the epidermis is ruptured with the development of a central depression and a number of radiating fissures. On the lower surface a brown crust is formed. The asci are in several layers. The cells between the asci present a filamentous appearance and soon gelatinise on contact with water. There is no foot for the stroma. Nor is there any differentiation into sterile and fertile regions. The ascospores are muriform. These characters indicate that the fungus should be classified under the Myriangiales in the genus *Ascomycetella* Ell.

(55) *Elsinoe mezoneuri* Ramakrishnan, T. S. and K., sp. nov.

Spots foliicolous, hypertrophied, bulged out towards the lower surface; stromata hypophyllous, dark violet, subepidermal, becoming erumpent, pseudoparenchymatous, covered by a dark layer of fungal cells, effuse; asci numerous, in two or three layers irregularly embedded in the stroma, broadly obovate, 21 × 16 $\mu$ (17–24 × 13–20); paraphyses absent; ascospores 4, hyaline, 1–3 septate, 13–15 × 4–7 $\mu$.

Maculæ in foliis; stromata hypophylla, fusco violacea, subepidermia, effusa; asci bis vel ter seriati, intus stromati infixi, hyalini, obovati, aparaphysati, 21 × 16 $\mu$ (17–24 × 13–20); ascosporidia 4, hyalina, primum uniseptata, postice 1–3 septata, fusiformia, 13–15 × 4–7 $\mu$.

On living leaves of *Mezoneurum cucullatum* W. and A. (Cæsalpinieæ), Anamalais, 30–1–1949, T. S. Ramakrishnan.

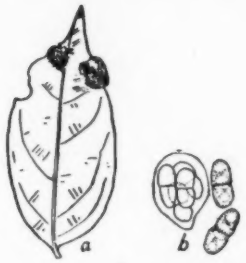


FIG. 5. *Elsinoe mezoneuri*, (*a*) leaf showing hypertrophy, (*b*) ascus and ascospores × 540.

The infected portion of the leaf can be easily recognised by the development of concavo-convex hypertrophied areas up to 1 cm. in diameter bulging out on the lower surface. The convex portion is coloured dark violet, owing to the formation of the stromata. The upper concave surface is light green in colour. One or more such galls may develop on a leaf. Most of the mature asci have only 4 ascospores clumped together in the centre of the ascus. The ascospores are usually two-celled with a constriction at the septum, but in some cases 2–3 septa were noticed. The imperfect stage was not observed.

(56) *Camarotella symploci* Ramakrishnan, T. S. and K., sp. nov.

Spots nil; stromata minute, 0·2–0·5 mm. diam., black, shining, amphigenous, isolated, sometimes clustered, usually uniloculate, subcuticular clypeate; loculi compressed-globose; asci basal, cylindrical with a very short foot, hyaline, 101 × 13 $\mu$ (85–143 × 11–15), paraphysate, paraphyses filiform; ascospores 8, brown, four-celled, constricted at the septa, tapering towards the ends, uniseriate, or irregularly arranged, 17–19 × 4–5 $\mu$.

Maculis nullis; stromata minuta, 0·2–0·5 mm. diam., nigra, micantia, amphigena, isolata vel gregaria, uniloculata, subcuticularia, clypeata; loculis subglobosis, compressis; asci cylindrici, brevipedicellati hyalini, paraphysati, paraphyses filiformes, 101 × 13 $\mu$ (85–143 × 11–15); ascosporidia 8, brunnea in maturitate, 4 cellata, constricata ad septa, utrinque attenuata, uniseriata vel irregulariter disposita, 17–19 × 4–5 $\mu$.

On living leaves of *Symplocos foliosa* W. (Symplocaceæ), Ootacamund, 24-3-1948, G. Rangaswamy and S. Damodaran.

*Dermatodothis javanica* Racib., *Homostegia symploci* Racib., *Phyllachora symploci* Pat., and *Cocconia placenta* (B. and Br.) Sacc. have been recorded on this host. Of these *Homostegia* alone has four-celled ascospores. But

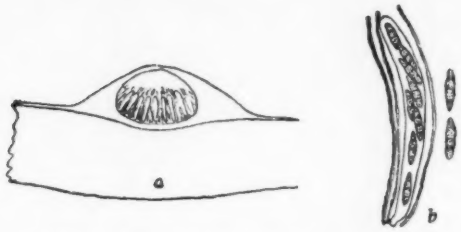


FIG. 6. *Camarotella symploci*, (*a*) section through stroma (diagrammatic), (*b*) ascus, paraphyses and ascospores × 250.

in this the stroma is immersed in the mesophyll while in the present fungus it is subcuticular. The structure of the fungus agrees with that of *Camarotella* Theiss. and Syd. Theissen and Sydow (1915) who have created this genus mention that the spores are hyaline. But the type specimen on which they created this genus is stated to be somewhat young. In the fungus under study the young spores are hyaline and they become coloured as they mature. So in spite of the presence of colour in the ascospores we have included it under *Camarotella* as it agrees with this genus in all other morphological features. In *Phyllachora* which is stated to possess only hyaline ascospores by Theissen and Sydow (*loc. cit.*), Stevens (1927) has included a number of species with coloured spores also. We feel that too much importance should not be attached to the colour of the spore in making generic distinctions.

(57) *Catacauma elæocarpi* Ramakrishnan, T. S. and K.

Ramakrishnan, T. S. and Ramakrishnan, K., *Proc. Ind. Acad. Sci.*, 1948, **28**, p. 57.

On living leaves of *Elæocarpus oblongus* Gærten. (Elæocarpaceæ), Ootacamund, 21–3–1949, T. S. Ramakrishnan.

This fungus was first recorded by us on *Elæocarpus munroii*, from Coonoor. The perfect stage on the present host is identical with the one observed on *E. munroii*. Associated with this are seen numerous stromata bearing pycnidia resembling those of the genus *Leptothyrium*. The formation of the stroma is similar to that of *C. elæocarpi*. The top of the stroma splits open and a definite ostiole is not seen. The conidiophores are arranged like a palisade. The conidia are one-celled, subhyaline or olivaceous, cylindric, straight or bent.

(58) *Plowrightia indica* Ramakrishnan, T. S. and K., sp. nov.

Spots amphigenous, circular, up to 0·5 cm. in diam., brown; stromata more often hypophyllous, sometimes amphigenous, subepidermal, erumpent, dark drown, arranged in circles, dothideoid; loculi immersed, ostiolate; asci hyaline, clavate, straight or slightly curved, short stalked, aparaphysate, 80 × 13 μ (64–94 × 12–15), wall gelatinising; ascospores 8, irregularly arranged, hyaline spindle-shaped, 1-septate, 37 × 4 μ (28–48 × 4).

Maculæ amphigenæ, orbiculares, usque 0·5 cm. diam., brunneæ vel fusco brunneæ; stromata prominenter hypophylla, interdum amphigena, subepidermalia, erumpentia, fusco-brunnea, circulariter disposita, dothideoidea; loculis immersis, ostiolatis; asci hyalini, clavati, recti vel leniter curvati, brevipedicellati, 80 × 13 μ (64–94 × 12–15), aparaphysati, pariete gelatiinoso; ascosporidia 8, irregulariter disposita, fusiformia, uniseptata, 37 × 4 μ (28–48 × 4).

On living leaves of *Jambosa læta* Bl. (Myrtaceæ), Anamalais, 30–1–1949, T. S. Ramakrishnan.

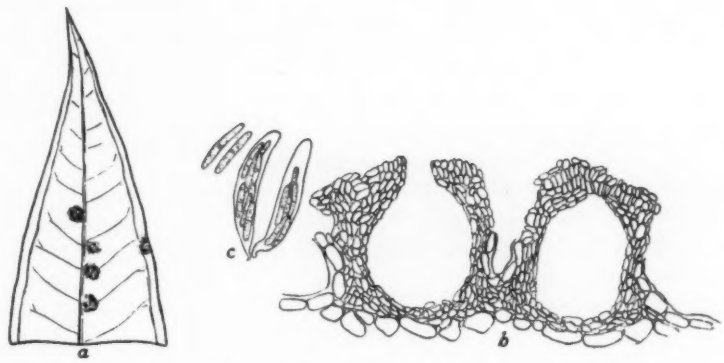


FIG. 7. *Plowrightia indica*, (*a*) leaf showing stromata, (*b*) section through stroma × 170, (*c*) asci and ascospores × 270.

One or more spots may develop on a leaf. The stromata appear as closely arranged, pulvinate, dark bodies arranged in circles. They are subepidermal and burst through the epidermis. The major portion of the stroma projects outside the leaf.

*Dothidella eugeniæ* (Thum.) Berl. and Vogl (Saccardo, 1891) and *Microcyclus labens* Sacc. and Syd. (Saccardo, 1905) have been recorded on *Eugenia* spp. But the fungus under study is entirely different from these.

(59) *Phyllachora sikkimense* Ramakrishnan, T. S. and K., sp. nov.

Infection spots amphigenous, small, 1–3 mm. diam.; stromata black, irregular, amphigenous, shining, pluriloculate, clypei above and below; locules somewhat flattened; asci cylindric, short stalked, 113 × 10 μ (102–127 × 8–13); paraphyses filiform, numerous; ascospores 8, uniseriate, fusiform, hyaline, one-celled, tapering towards both ends, 24 × 6 μ (21-30 × 4–6·5).

Maculæ amphigenæ, parvæ, 1–3 mm. diam.; stromata nigra, irregularia, amphigena, micantia, pluriloculata, clypei infra et supra; loculi compressi; asci hyalini, brevipedicellati, 113 × 10 μ (102–127 × 8–13); paraphyses filiformes; ascosporidia 8, uniseriata, fusiformia, hyalina, unicellata, utrinque attenuata, 24 × 6 μ (21–30 × 4–6·5).

On living leaves of *Canarium sikkimense* King. (Burseraceæ), Anamalais, 30–1–1949, T. S. Ramakrishnan.

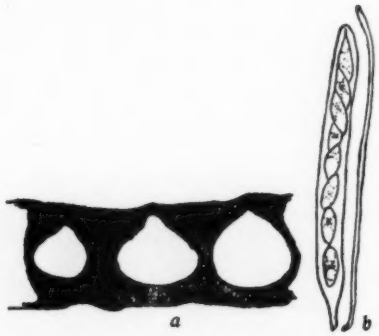


FIG. 8. *Phyllachora sikkimense*, (*a*) stroma with locules (diagrammatic), (*b*) Ascus and paraphyses × 400.

*Phyllachora canarii* P. Henn has been recorded on *Canarium luzonicum* from the Philippines (Saccardo, 1913; Theissen and Sydow, 1915). The present fungus differs from this in having much larger asci and ascospores.

(60) *Sphærodothis borassi* Ramakrishnan, T. S. and K., sp. nov.

Spots amphigenous, elliptic, up to 1 cm. in length, and 0·6 cm. in breadth, isolated or confluent, brown; stromata amphigenous, isolated or confluent, unilocular, many in a spot; loculi deep seated, compressed; asci hyaline, clavate, straight or flexuous, 93 × 20 μ (66–126 × 15–30); ascospores 8, one-celled, irregularly arranged, reddish brown, fuscoid, sometimes curved, wall minutely reticulate, 30 × 11 μ (21–39 × 6–15).

Maculis amphigenis, ellipticis, usque 1 cm. longis et 0·6 cm. latis, isolatis vel confluentis, brunneis; stromatibus nigris, amphigenis, isolatis vel confluentis, unilocularibus, numerosis; loculis compressis, profunde immersis; asci hyalini, clavati, recti vel flexuosi, 93 × 20 μ (66–126 × 15–30); ascosporidia 8, unicellata, irregulariter disposita, fusioidea, interdum curvata, rubro-brunnea, 30 × 11 μ (21–39 × 6–15), episporio minute reticulato.

On living leaves of *Borassus flabellifer* L. (Palmaceæ) Coimbatore, 11-4-1949, K. Ramakrishnan.

FIG. 9. *Sphærodothis borassi*, (*a*) stroma (diagrammatic) × 45, (*b*) asci and ascospores × 270.

Numerous spots are formed on the leaves. In each spot varying numbers of black stromata are present. The spores are prominently coloured and when viewed under the oil immersion lens present a finely reticulated epispore.

(61) *Bubakia indica* Ramakrishnan, T. S. and K., sp. nov.

Pycnia on young leaves, gregarious, minute, amphigenous, subcuticular, without ostiolar paraphyses, reddish brown; æcia not seen; uredia rare, mixed with telia, subepidermal, hypophyllous; urediospores globose to obovate, with short broad stalks, echinulate, subhyaline, 27 × 18 μ (20–34 × 14–24); telia subepidermal, numerous, hypophyllous, crustaceous; teliospores sessile, one-celled, 4–5 layered, not catenulate, polygonal, thin-walled, subhyaline, slightly thickened at the apex, germinating immediately 26 × 18 μ (17–37 × 10–24); basidium stout, recurved, four-celled, basidospore globose, hyaline.

Pycnia amphigena, minuta, gregaria, subcuticularia, rubrobrunnea, aparaphysata; æcia ignota; uredia rara, teliis immixta, subepidermalia, hypophylla; uredosporis globosis vel obovatis, brevi vel lati pedicellatis, echinulatis, subhyalinis, 27 × 18 μ (20–34 × 10–24); telia subepidermia, numerosa, hypophylla, crustacea; teliosporis sessilibus, unilocularibus, 4–5 stratosis, non-catenulatis, polygonis, subhyalinis, membranis tenuis 26 × 18 μ (17–37 × 10–24), apice leniter incrassatis, statim germinantibus; basidiis 4 cellatis, crassis, recurvatis, sporidiis globosis, hyalinis.

On living leaves of *Glochidion neilgherrense* W. (Euphorbiaceæ), Dodabetta, Niligiris, 17–1–1949, T. S. Ramakrishnan.

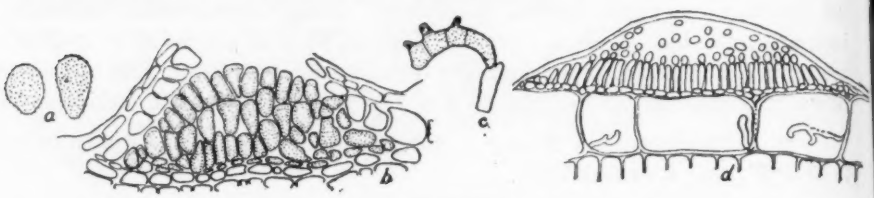


FIG. 10. *Bubakia indica*, (*a*) urediospores, (*b*) telium, (*c*) germination teliospores, (*d*) pycnium × 270.

The pycnia are formed in groups on yellowish infection spots on young leaves and twigs. The uredia and telia are observed on older leaves hypophyllously with corresponding discolouration on the upper surface. Paraphyses or peridia are absent. The telia may sometimes develop on old uredia. The teliospores are not catenulate, but are found closely wedged in between one another. The spores easily separate when the telia are crushed. The spores in the uppermost layer germinate *in situ* giving a whitish powdery appearance to the telia.

*Schræteriaster glochidii* Syd. and *Phakopsora formosana* H. and P. Syd. have been recorded on *Glochidion zeylanicum* and *G. fortunei* respectively from Formosa (Saccardo, 1925). *Bubakia glochidii* (Syd.) Diet. has been described on *Glochidion psidioides* and *Glochidion* Sp. from the Philippines (Arthur and Cummins, 1936). The rust under study possesses subcuticular pycnia. The urediospores have very short stalks almost appearing sessile. The uredia and telia are subepidermal. These indicate that the rust is a *Bubakia* (Mundkur, 1943). The descriptions of the sori of the former rusts do not agree with those of the one under study.

(62) *Phakopsora kirganeliæ* Ramakrishnan, T. S. and K., sp. nov.

Rust spots amphigenous, reddish brown, circular; pycnia, æcia and uredia not seen; telia hypophyllous, minute, subepidermal, erumpent; teliospores one-celled, rectangular or globose, sessile, thin-walled, subhyaline, smooth, many layered, germinating *in situ*, 17 × 9 $\mu$ (12–24 × 6–12); basidia four-celled, hyaline, curved, basidiospores hyaline, globose.

Maculæ amphigenæ orbiculares, rubrei brunnei colores; pycnia æcia et uredia ignota; telia hypophylla, minuta, subepidermalia erumpentia; teliosporis unicellatis, rectangularibus vel globosis, sessilibus, 3–4 stratosis *in situ* statim germinantibus, 17 × 9 $\mu$ (12–24 × 6–12), pariete levi, tenui; basidia 4 cellata, hyalina, curvata, sporidiis hyalinis, globosis,

On living leaves of *Kirganelia reticulata* Baill. (Euphorbiaceæ), Coimbatore, 1-1-1949, T. S. Ramakrishnan.

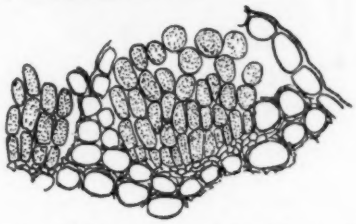

FIG. 11. *Phakopsora kirganeliæ*, telia × 250.

The rust spots are prominent. On the lower surface of the spot the telia are closely arranged and when the spores germinate appear as white, crowded powdery sori. The teliospores rarely appear in vertical rows, but are more often irregularly wedged in between one another.

*Phakopsora phyllanthi* Diet. has been recorded on *Phyllanthus distichus* (Saccardo, 1912; Butler and Bisby, 1931). The rust under study differs from this in the method of formation of the sori and other characters.

(63) *Uromyces piauhyensis* P. Henn.

Sydow, H. and P. *Monogr. Ured.* 1910, **2**, p. 12.

On living leaves of *Wedelia urticæfolia* DC. (Compositæ) Perur (Coimbatore), 1-1-1949, T. S. Ramakrishnan and K. Ramakrishnan.

Uredia and telia are present. The characteristic shape and papilla of the teliopspores are useful in identifying the species.

(64) *Cylindrosporium dichanthii* Ramakrishnan, T. S. and K., sp. nov.

Spots amphigenous, elongated, 1 × 0·5 cm., salmon pink in colour; acervuli mostly hypophyllous, subepidermal, crowded, orange coloured, hemispherical with a flattened top, conidiophores short, subhyaline; conidia acicular, curved, continuous, hyaline, 32 × 1·8 $\mu$ (25–43 × 0·9–4).

Maculæ amphigenæ elongatæ, 1 × 0·5 cm., salmonicolores; acervuli prominenter hypophyllis, crebremis, subepidermalibus, luteo colores, hemisphericis, apice truncatis; conodiophoris brevis, subhyalinis; conidiis acicularis, curvatis, continuis, hyalinis, 32 × 1·8 $\mu$ (25–43 × 0·9–4).

On living leaves of *Dichanthium annulatum* Stapf. (Gramineæ) Coimbatore, 1-3-1949, T. S. Ramakrishnan.

The acervuli are crowded and invariably developed under the lower epidermis which is raised and comes off easily in the later stages exposing

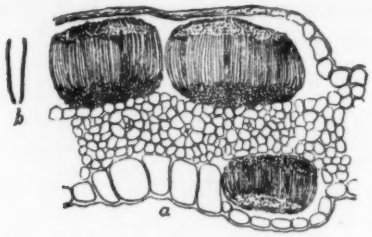


FIG. 12. *Cylindrosporium dichanthii*, (*a*) section showing acervuli, (*b*) conidia ×170.

the bright orange coloured acervuli. Each acervulus has a plectenchymatous base made up of small fungal cells. From the upper surface of this plectenchyma numerous slender spores are produced which remain together in a mass. The mass is flattened or slightly concave at the top.

(65) *Septogloeum feroniæ* Ramakrishnan, T. S. and K., sp. nov.

Spots hypophyllous, minute, isolated, confluent, brown, slightly depressed; acervuli black, in the centre of the spot, subepidermal, erumpent; conidia straight or flexuous, cylindric to obclavate, 1–4 septate, subhyaline to light olivaceous, conidia 33 × 4 $\mu$ (24–45 × 3–6); conidiophores short.

Maculis hypophyllis, minutis, brunneis, isolatis vel confluentis, leniter depressis; acervulis atris, hypophyllis, subepidermalibus, erumpentibus; conidiis cylindricis vel obclavatis, rectis vel flexuosis, 1–4 septatis, subhyalinis vel dilute olivaceis, 33 × 4 $\mu$ (24–45 × 3–6); conidiophoris brevis.

On living leaves of *Feronia elephantum* Corr. (Rutaceæ), Perur, (Coimbatore), 1–1–1949, T. S. Ramakrishnan and K. Ramakrishnan.

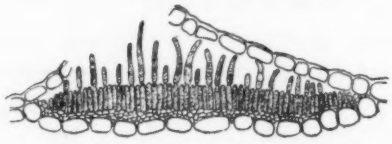

FIG. 13. *Septoglæum feroniæ*, acervulus × 170.

The spots are very small. One or more acervuli are seen in the centre of each spot.

(66) *Diplodia longipedicellata* Ramakrishnan, T. S. and K., sp. nov.

Spots indefinite, amphigenous, pale green; pycnidia epiphyllous, deep seated, very irregular in shape, grouped in the spot, up to 15 in each group, ostiolate; pycnidiospores two-celled, upper cell bigger, lower cell smaller, elliptic, obovate or pyriform, dark olive brown, 13 × 10 $\mu$ (10–14 × 7–12),

stalked, stalks hyaline, up to 50 $\mu$ in length, pushed out through the ostiole in long black columns (sporehorns).

Maculis indistinctis, amphigenis, pallide virides; pycnidiis amphigenis, profunde immersis, irregularibus, gregariis in maculis, ostiolatis; conidiis initio hyalinis postice fusco-olivaceo-brunneis, 2-cellatis, cellulis inequalis, ellipticis, obovatis vel pyriformibus, 13 × 10 $\mu$ (10–14 × 7–12), in massis nigris et filiformibus, pedicellatis, pedicellis hyalinis, usque 50 $\mu$ longis.

On living leaves of *Syzigium montanum* Gamb. (Myrtaceæ), Ootacamund, 20-3-1949, T. S. Ramakrishnan.

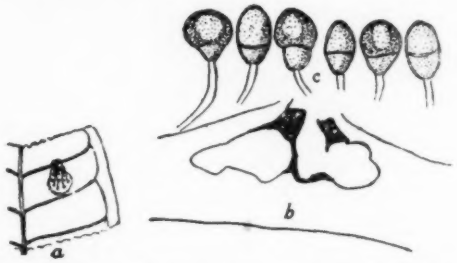


FIG. 14. *Diplodia longipedicellata*, (*a*) portion of leaf with spore horns (*b*) pycnidia, (*c*) pycnidiospores × 570.

Pale green thickened spots are present on the leaves. On both sides of the spots black hair like colums of spores are observed issuing from the ostioles. The spores are peculiar in that a majority of them have unequal cells, the upper cell being larger and the lower much smaller. The stalks are persistent and the colums are formed by the interlacing of the stalks. The pycnidia are deep seated, irregular in shape and may be isolated or confluent.

We are thankful to Dr. Mukerji, Curator, Royal Botanic Gardens, Calcutta, for kindly identifying some of the host plants.

## REFERENCES

Arthur, J. C. and Cummins, G. B. .. *The Philippine J. Sci.*, 1936, **61**, 474.
Butler, E. J. and Bisby, G. R. .. *The Fungi of India*, 1931, 62.
Mhatre, J. R. and Mundkur, B. B. .. *Lloydia*, 1945, **8**, 131–38.
Mundkur, B. B. .. *Mycologia*, 1943, **35**, 539.
Saccardo, P. A. .. *Syll. Fung.* 1891, **9**, 1038.
———— .. *Ibid.*, 1905, **17**, 845.
———— .. *Ibid.*, 1912, **21**, 608.
———— .. *Ibid.*, 1913, **22**, 411.
———— .. *Ibid.*, 1925, **23**, 841–42.
———— .. *Ibid.*, 1926, **24**, Sect 1, 570–71.
———— .. *Ibid.*, 1928, **24**, Sect. 2, 797.
Stevens, F. L. .. *Ill. Biol. Monogr.*, 1927, **11**, No. 2, 1–103
Theissen, F., and Sydow, H. .. *Ann. Mycol.*, 1915, **13**, 213, 369, 547, 570 & 606.

# BACTERIOLOGICAL SURVEY OF SEA-WATER FROM THE COAST OF MADRAS CITY (BAY OF BENGAL)*

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Bacteria are widely distributed in the marine environment. They influence the chemical and biological conditions in the sea. Probably there are as many, or even more, genera of bacteria present in the sea as in the terrestrial environment. Recent work on marine bacteriology lays increasing emphasis on the chemical activities of the bacteria. Bacterial degradation of organic matter into inorganic salts which support plant life is an essential step in the maintenance of the fertility of the ocean. The interconversion of the salts of nitrogen, the transformation of the compounds of phosphorus and sulphur, the breakdown of cellulose, chitin, etc., are processes more or less influenced by microbial action (Waksman, *et al.*, 1933 *a*, 1933 *b*, 1933 *c*; Hock, 1940; Brand, *et al.*, 1937–42). The role of marine bacteria in the formation of petroleum, which occurs in ancient marine sediments, may prove to be significant (Zobell, 1946 *b*, 1947 *a*, 1947 *b*). The dissolved organic matter in the sea, which is otherwise unavailable, is utilised by the bacteria for the synthesis of their cells, which again provide particulate food material for certain marine animals (Zobell and Landon, 1937). The role of marine bacteria in the spoilage of fish and other marine food products is also of practical importance.

A large volume of work has been done by marine bacteriologists abroad, particularly in the U.S.A. and in the U.S.S.R.; but there is paucity of data for tropical waters. The influence of bacterial activity on the fertility of the sea which is particularly low in the tropical regions, is of fundamental importance. A survey of the occurrence, distribution and nature of the bacteria responsible for the different biochemical processes is essential for understanding their relative influences on the fertility of the sea. The present investigations are in relation to these aspects, *i.e.*, (1) seasonal variation in the density of the bacterial population of surface sea water, (2) relative abundance of bacteria in (i) sea water and (ii) in association

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with plankton, (3) qualitative and quantitative survey of the groups of marine bacteria of biochemical significance.

*Procedure.*—Bacterial populations were evaluated using the plating procedure. Surface sea water samples were collected three miles off the shore (Madras Marina) in sterile glass bottles and plated out immediately upon arrival at the laboratory (about one hour). Plankton samples were obtained at the same time, hauling nets approximately equal distances during all the collections; these samples were placed in sterile glass bottles for transport to the laboratory. Sea water agar (Medium 2216, Zobell, 1946 *a*) was used as the plating medium. Sea water was plated in 1 c.c., and 1 in 10 dilution duplicates. Plankton samples were plated in 1 in 100 and 1 in 1000 dilution duplicates. Filtered and autoclaved sea water was used for the dilution blanks. The colonies present after 48 hours' incubation at 25° C. were counted, using a magnifying glass. It is possible that organisms which grow very slowly will be excluded by this procedure, but longer periods of incubation cause inaccuracy in counting due to the presence of colonies of spreading nature. Workers abroad have used lower temperatures and longer periods of incubation, but it may be noted that their studies were generally restricted to relatively colder regions.

## Physiological Groups of Marine Bacteria

*Procedure.*—Samples of sea water and plankton were inoculated in 1 c.c., 0·1 c.c., 0·01 c.c. lots into appropriate selective media. "Aged" sea water (Zobell, 1946 *a*) was used for the preparation of the media. This procedure, besides the qualitative demonstration of the active bacteria, also indicates the order of numerical magnitude of their occurrence.

*Nitrate reducers* were present in large numbers throughout the period of investigation.

*True denitrifiers*, i.e., bacteria which liberate free nitrogen from nitrogenous salts, were absent.

Bacteria capable of growing in sea water media containing carbohydrates, but free from any added nitrogen compounds, were usually found in 10 c.c. samples of sea water.

*Nitrifying bacteria.*—Samples of sea water failed to give a positive nitrite reaction during the period of incubation (eight weeks). Plankton samples, when inoculated into ammonium sulphate medium, always produced nitrite within one to two weeks' incubation at room temperature. Bacteria capable of oxidising "ammonium" to "nitrite" appear to be present constantly in association with plankton. In view of the possibility

*Density of physiological groups of bacteria during different seasons*

| Period | Nitrate Reducers per c.c. of sea water | Nitrogen fixers of sea water per 10 c.c. | Denitrifiers per 10 c.c. of sea water |
|---|---|---|---|
| July 1948–Nov. 1948 | 100 to 1,000 | 1 to 10 | nil |
| Dec. 1948–March 1949 | over 1,000 | 1 to 10 | nil |
| April 1949–July 1949 | 100 to 1,000 | 1 to 10 | nil |

| Period | Nitrifying bacteria per c.c. of | | Urea fermenters per c.c. of sea water |
|---|---|---|---|
| | Sea water | Plankton | |
| July 1948–Nov. 1948 | nil | 1 to 10 | 1 to 10 |
| Dec. 1948–March 1949 | nil | 10 to 100 | 10 to 100 |
| April 1949–July 1949 | nil | 1 to 10 | 1 to 10 |

| Period | Cellulose fermenters per c.c. of | |
|---|---|---|
| | Sea water | Plankton |
| July 1948–Nov. 1948 | nil | 1 to 10 |
| Dec. 1948–March 1949 | nil | 1 to 10 |
| April 1949–July 1949 | nil | 1 to 10 |

of plankton being an important site for the activity of marine bacteria, this nitrification by bacteria associated with plankton merits a detailed investigation (Carey, 1938).

There appears to be no seasonal qualitative change in the biochemically active bacterial flora, the changes observed being of a quantitative nature only.

Study of the density of the bacterial population of surface sea water off Madras during the period April 1948 to July 1949 was carried out. During April 1948 to July 1948 bacteria averaged 400 per c.c.; August 1948 to November 1948, 600 per c.c.; during December 1948 to March 1949 the average was over 1,000 per c.c. From the middle of March 1949 a sharp fall was noted. The level of bacterial population in April, May

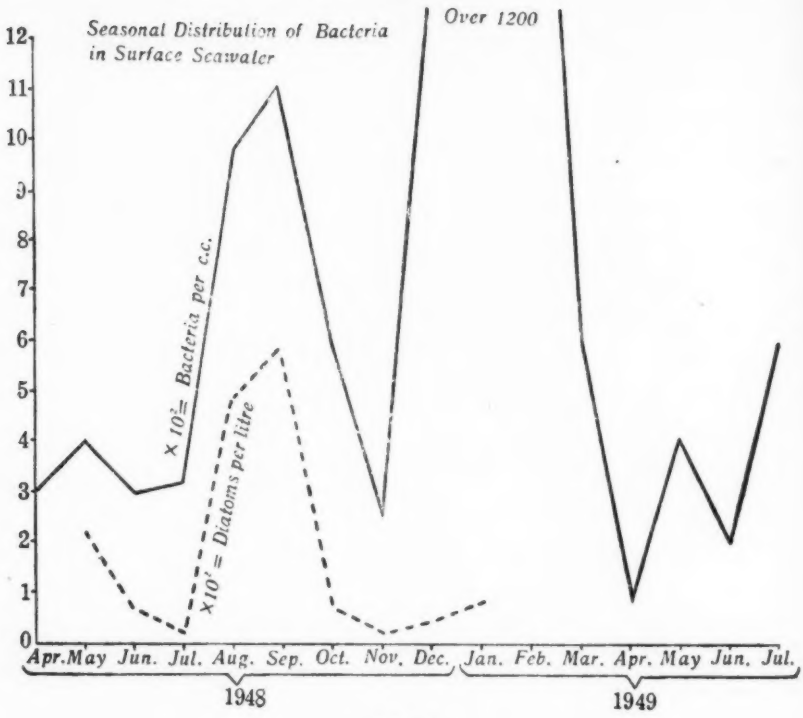
| Month | Bacteria per c.c. of sea water (Monthly average) | Bacteria per c.c. of sea water | | Bacteria per c.c. of plankton | | Ratio of Bacteria per c.c. of plankton / Bacteria per c.c. of sea water | | Remarks |
|---|---|---|---|---|---|---|---|---|
| | | Min. | Max. | Min. | Max. | Min. | Max. | |
| April 1948 .. | 312 | 220 | 400 | .. | .. | .. | .. | |
| May 1948 .. | 366 | 100 | 560 | 10,000 | 1,300,000 | 150 | 2,350 | |
| June 1948 .. | 413 | 220 | 870 | 3,700 | 6,800,000 | 29 | 16,000 | |
| July 1948 .. | 300 | 240 | 340 | 50,000 | 5,700,000 | 166 | 19,000 | |
| Aug. 1948 .. | 820 | 260 | 1,200 | 15,000 | 500,000 | 20 | 416 | Ratio has |
| Sept. 1948 .. | 1,110 | 290 | 1,800 | 25,000 | 5,000,000 | 200 | 5,300 | diminished |
| Oct. 1948 .. | 650 | 200 | 1,000 | 40,000 | 2,300,000 | 50 | 4,107 | |
| Nov. 1948 .. | 500 | 220 | 1,000 | 5,000 | 150,000 | 5 | 680 | Ratio low |
| Dec. 1948 .. | 1,200 | 1,000 | 2,110 | 10,000 | 200,000 | 7 | 215 | Ratio low |

| Month | Bacteria per c.c. of sea water (Monthly average) | Bacteria per c.c. of sea water | | Bacteria per c.c. of plankton | | Ratio of Bacteria per c.c. of plankton / Bacteria per c.c. of sea water | | Remarks |
|---|---|---|---|---|---|---|---|---|
| | | Min. | Max. | Min. | Max. | Min. | Max. | |
| Jan. 1949 .. | 1,500 | 900 | 2,000 | 6,500 | 50,000 | 6 | 33 | Low ratio throughout this month |
| Feb. 1949 .. | 1,560 | 700 | 2,500 | 15,000 | 82,000 | 6 | 206 | do |
| March 1949 .. | 660 | 150 | 1,600 | 40,000 | 120,000 | 89 | 390 | Low ratio. Sharp fall in bacterial population after 2nd week of March |
| April 1949 .. | 460 | 70 | 1,280 | 15,000 | 500,000 | 110 | 5,000 | |
| May 1949 .. | 390 | 70 | 800 | 15,000 | 1,000,000 | 50 | 5,000 | Ratio has increased |
| June 1949 .. | 200 | 40 | 490 | 20,000 | 380,000 | 200 | 1,630 | Ratio high |
| July 1949 .. | 650 | 70 | 2,000 | 40,000 | 112,000 | 140 | 1,244 | do |

and June 1949 was the same as observed for the previous year, indicating the possibility of the variation being seasonal. Observations over a longer period are necessary.

During April 1948 to November 1948 a close parallel between the abundance of bacteria and phytoplankton is observed (Graph I). This parallel does not continue in the next few months. The latter period was characterised by the prevalence of continuous "disturbed" conditions, which might account for the discontinuity (see the following).

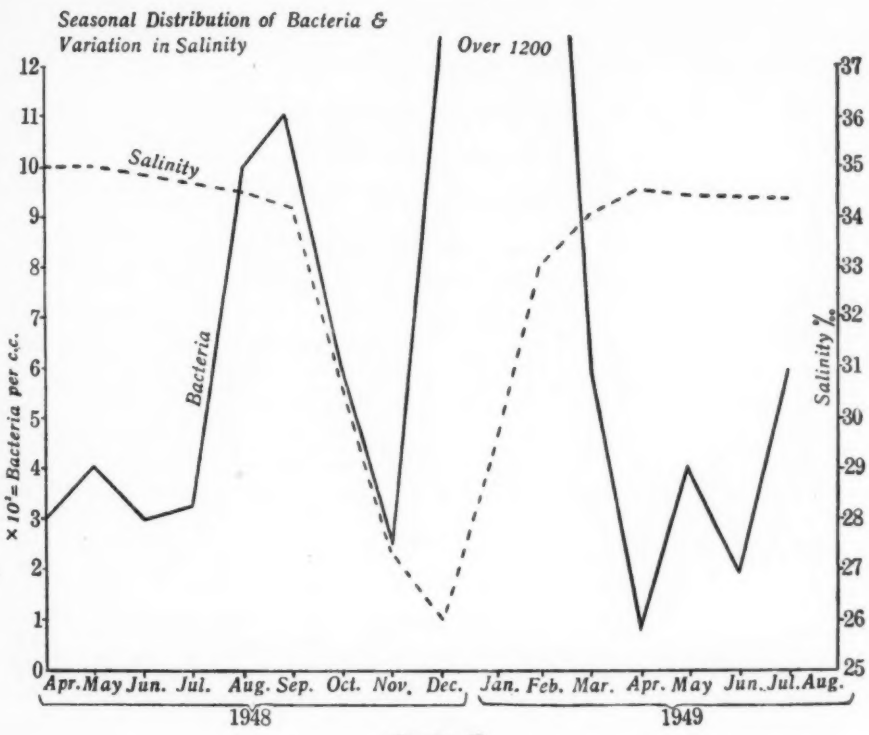
The bacteria in the plankton tow are thousands of times higher than in the sea water, the ratio fluctuating widely from 5:1 to 19,000:1. The ratio tends to be low during periods of stormy weather. Higher bacterial counts in plankton samples are usually obtained when the plankton




GRAPH I

consists predominantly of phytoplankton and lower counts when zooplankton predominates. The nature of the influence of the plankton on bacterial population is not clear, though it is generally believed that the phytoplankton secretes nutrients for the bacteria, while the zooplankton may diminish the bacterial population by grazing.

During and immediately following stormy weather the bacterial counts were always high. This may be ascribed to the upwelling of particulate organic matter by the disturbed conditions. The numerical magnitude of the bacterial population appears to bear a relation to the salinity of the sea water (Graph II). The curves, showing the monthly average density of bacterial population and the monthly average salinity do not show a strictly inverse relation, but the tendency of the bacterial population to rise as the salinity falls and to diminish as the salinity approaches the normal is obvious. It is unlikely, however, that the salinity changes as such would influence the bacterial population, since marine bacteria are known to




GRAPH II

tolerate the salinity ranges in question. Regarding the fall in salinity as an index of the influx of masses of fresh water carrying organic matter might provide an explanation for the rise in bacterial population.

During the four months November 1948 to February 1949 comparatively lower values for the concentration of nitrate in the sea water were reported (private communication by Mr. R. Jayaraman, Central Marine Fisheries Research Station, Mandapam). The bacterial population during this period was the maximum observed. The lowering in the nitrate level may have a relation to the high bacterial population, since many of the bacteria are nitrate reducers.

My thanks are due to Dr. (Mrs.) F. Thivy, Central Marine Fisheries Research Station, Mandapam, for kindly making the phytoplankton counts.

## References


1. Brand, Theodor von and Rakestraw, Norris, W. .. *Biol. Bull.*, 1937–42, **72**, 77, 79, 81 and 83.
2. Carey, Cornelia, L. .. "The occurrence and distribution of nitrifying bacteria in the sea," *Journ. Mar. Res.*, 1938, **1**, 291.
3. Hock, C. W. .. "Decomposition of chitin by marine bacteria," *Biol. Bull.*, 1940, **79**, 199.
4. Waksman, Selman, A. *et al.* .. "Bacteriological examination of sea water and marine bottoms," *ibid.*, 1933*a*, **64**, 183.

   ———— .. "Decomposition of marine plant and animal residues by bacteria," *ibid.*, 1933*b*, **64**, 57.

   ———— .. "Bacteria concerned in the cycle of nitrogen in the sea," *ibid.*, 1933*c*, **65**, 137.
5. Zobell, C. E. and Landon, W. A. .. "Bacterial nutrition of the California sea mussel" *Proc. Soc. Exp. Biol. & Med.*, 1937, **36**, 607.
6. Zobell, C. E. .. "*Marine Microbiology*," (A monograph on hydrobacteriology), Published by the Chronica Botanica Company, Waltham, Mass., U. S. A., 1946 *a*.

   ———— .. "Function of bacteria in the formation and accumulation of petroleum," *Oil Weekly*, 1946*b*, **120** (12), 30.

   ———— .. "Microbial transportation of molecular hydrogen in marine sediments," *Bull. Amer. Assm. Petrol. Geol.*, 1947*a*, **31**, 1709, 51.

   ———— .. "Bacterial release of oil from oil-bearing materials," *World Oil*, 1947*b*, **126** (13), 36 ; **127** (1), 35.

# STUDIES ON THE ANATOMY OF THE TAIL IN SAURIA AND RHYNCHOCEPHALIA*

## IV. *Anguis fragilis*

By Syed Muzammil Ali, Ph.D.

Received December 8, 1949
(Communicated by Prof. Beni Charan Mahendra, D.Sc., F.Z.S., F.A.Sc.)

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### 1. Introduction

As our knowledge about the structure of the tail in limbless lizards is limited so far only to a note by Boulenger (1888) on the lepidosis of *Ophisaurus* and a brief observation by Woodland (1920) on the presence of perforations in the cartilaginous tube in regenerated tails of *Pygopus*, it was thought desirable to select *Anguis fragilis* for a detailed study. This type of tail, although adapted for a fossorial life, is really a modification of the autotomous one, the principal difference being due to configuration. The facts that it is round in cross-section, uniformly thick throughout and of the same calibre as the trunk can be explained as the result of the borrowing habit.

The tail of this lizard is rather long as compared to that of other burrowing reptiles, nearly circular in transverse sections, uniformly thick throughout its extent, and bluntly terminated. It scarcely differs from the rest of the body in its superficial appearance, and passes on in front into the trunk almost indistinguishably.

As the specimen studied by me had, at the end of the tail, a regenerated piece $\frac{3}{8}$ inch long, I have been able to study the peculiarities of regeneration as well.

* Part of a thesis approved for the Ph.D. degree in Agra University.

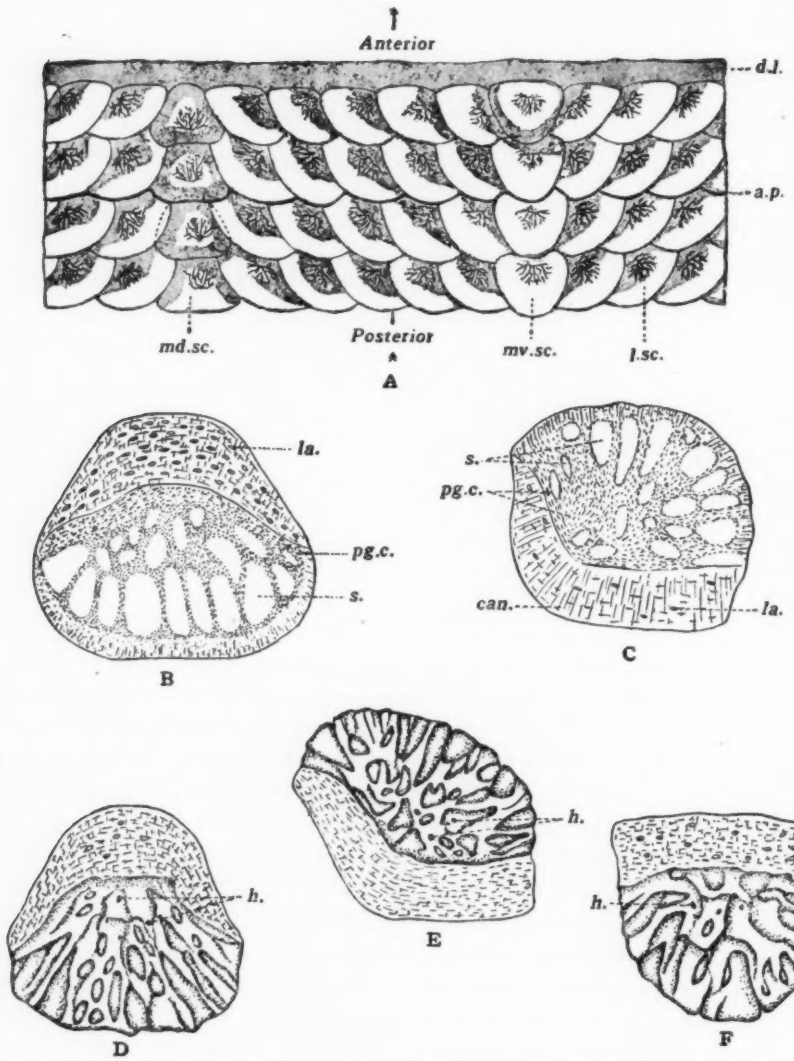


FIG. 1. (*A*) A piece of the caudal skin of *Anguis fragilis*, cut along the lateral line and spread out to show the normal scalation (× 4); (*B*) Dorsal and (*C*) Lateral caudal scales seen under the microscope (× 12); (*D*) Dorsal, (*E*) Lateral, and (*F*) Ventral caudal scales, stained with alizarin and seen under the microscope (× 12).

*a.p.*, autotomy plane; *can.*, canaliculæ; *d.l.*, dermal ledge; *h.*, pore; *la.*, lacunae; *l.sc.*, lateral scales; *md.sc.*, mid-dorsal scales; *mv.sc.*, midventral scales; *pg.c.*, pigment cells; *s.*, spaces devoid of pigment.

## II. Epithelium and Scalation

While the general structure of the epithelium resembles that of other lizards, a tough membrane is present in *Anguis fragilis* underneath the skin. This appears to be dermal in origin and covers the caudal musculature. When removed, it comes off in the form of annular pieces breaking at the autotomy planes and corresponding to two transverse rows of caudal scales.

The tail is covered by imbricate scales forming transverse annular rows, two rows together constituting a single autotomy segment. When the caudal skin breaks off at a plane of cleavage, the front end of the posterior piece (Fig. 1, A) is seen to have a projecting dermal ledge, which was, in the intact condition, covered over by the posterior edge of the anterior piece. The plane of epithelial cleavage is not straight, but oblique.

The scales are arranged obliquely. Almost all of them are roughly oval in outline, except that the median dorsals and median ventrals are heart-shaped. In the former (Fig. 1, B) the broad edges are situated behind and the anterior and lateral borders are overlapped by the adjacent scales, while in the latter it is the narrow edge that is posterior and the posterior borders cover part of the neighbouring scales. On account of such a disposition, almost the whole of a median ventral is exposed and visible, but a median dorsal is partially covered both anteriorly and laterally by the adjoining scales. The difference in the disposition of the median ventrals from that of the median dorsals has not been hitherto observed. It can be regarded as the result of perfect regularity in imbrication of the caudal scales, provided we assume that the overlapping starts from the mid-ventral line and passes laterally upwards on both sides, meeting above at the mid-dorsal row.

The *lateral* scales (Fig. 1, C) are almost oval in outline, and are arranged to form both oblique and transverse rows (Fig. 1, A) in such a way that their postero-lateral borders cover the antero-lateral ones of the scales of the succeeding row. There are five laterals on each side, the total number of scales in each transverse row being twelve; *i.e.*, one median dorsal, one median ventral, and ten laterals.

An examination of the scales under the low powers of a microscope (Fig. 1, B and C) reveals numerous pigment cells scattered extensively in an irregular manner, leaving a few oval or round regions unpigmented. Pigment cells are either absent or sparsely present on the unexposed borders of a scale where such bony structures as lacunæ, lamellæ and canaliculæ are visible. Besides these, in alizarin-stained scales (Fig. 1, D-F) a number

of pores are seen disposed obliquely or in a straight direction, and the unpigmented regions appear like globular cavities faintly stained.

The last normal row preceding the regenerated region (Fig. 2) consists of scales which are somewhat longer and larger than the others and are regularly arranged. Behind these the scales which are regenerated are more or less irregular in shape, although they are arranged in a manner recalling the normal order. Still posteriorly, however, the lack of uniformity in size and outline, as well as the irregularity in arrangement, becomes extremely striking. The end of the tail is encased in a conical scute with an uneven margin.

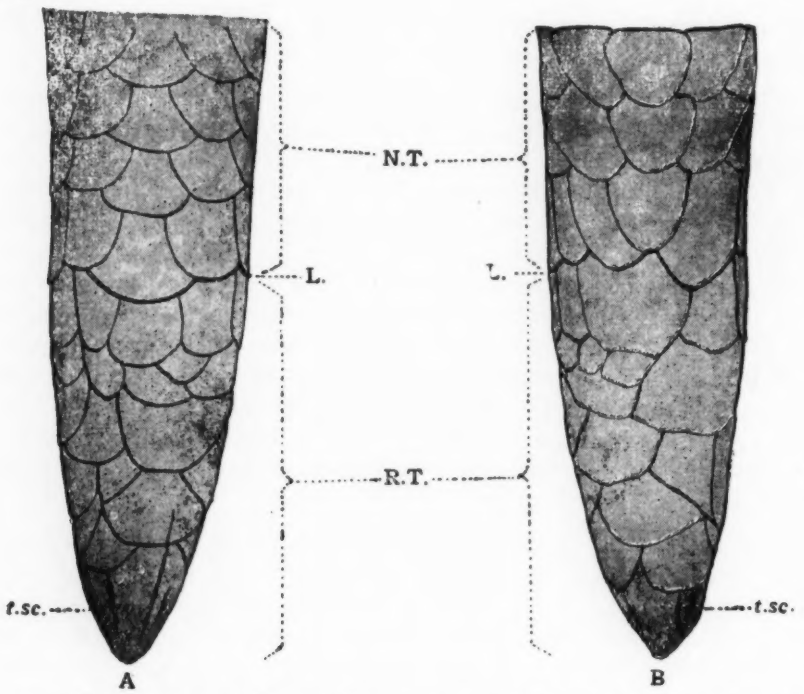


FIG. 2. (*A*) Dorsal and (*B*) ventral views of the tail of *Anguis fragilis*, showing both the normal and regenerated regions (× 6).

*L.*, line between the normal and regenerated tail; *N.T.*, normal tail; *R.T.*, regenerated tail; *t.sc.*, terminal conical scute.

## III. THE CAUDAL VERTEBRÆ

The vertebræ (Fig. 3) are slender in build and more elongated than in most lizards. A conspicuous, though not stout, neural spine is present,

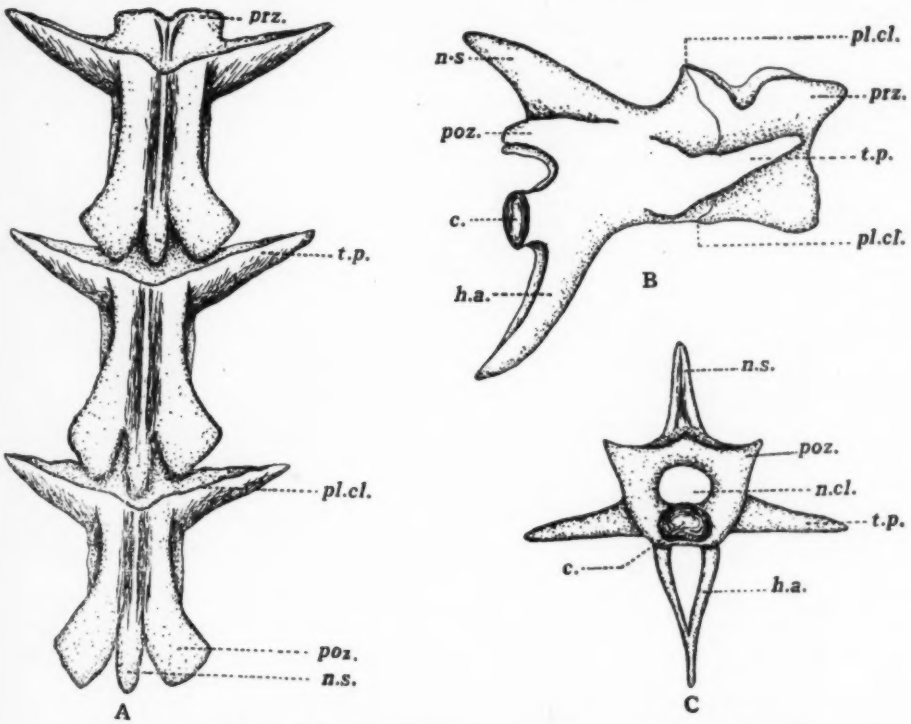


FIG. 3. (*A*) Dorsal view of three caudal vertebrae of *Anguis fragilis*; (*B*) Lateral and (*C*) posterior views of a caudal vertebra (× 12).

*c.*, centrum; *h.a.*, haemal arch; *n.cl.*, neural canal; *n.s.*, neural spine; *pl.cl.*, plane of cleavage; *poz.*, postzygapophysis; *prz.*, prezygapophysis; *t.p.*, transverse process.

directed backwards, and the articular processes are well developed. The prezygapophyses are flat facets, facing upwards and inwards. The postzygapophyses are broad and wing-shaped, facing downwards and outwards. The medium-sized transverse processes lie at about the middle height of the vertebra on each side, have broad bases, and are directed outwards, forwards and somewhat upwards. The hæmapophyses are sword-shaped, projecting from the base of the centrum near its posterior end and directed downwards and backwards to meet each other ventrally to enclose the *V*-shaped hæmal canal.

The plane of cleavage (Fig. 3, *B*) which passes not in front of, but through the bases of the transverse processes, divides the vertebra into two unequal halves. The anterior half, the shorter of the two, bears the pre-

zygapophyses and the bases of the transverse processes, while the posterior one, which is elongated, bears the neural spine, the postzygapophyses, the distal parts of the transverse processes and the hæmapophyses.

Transverse sections of the vertebræ passing through the autotomy plane (Fig. 4, *A*) differ in several respects from those passing through the inter-

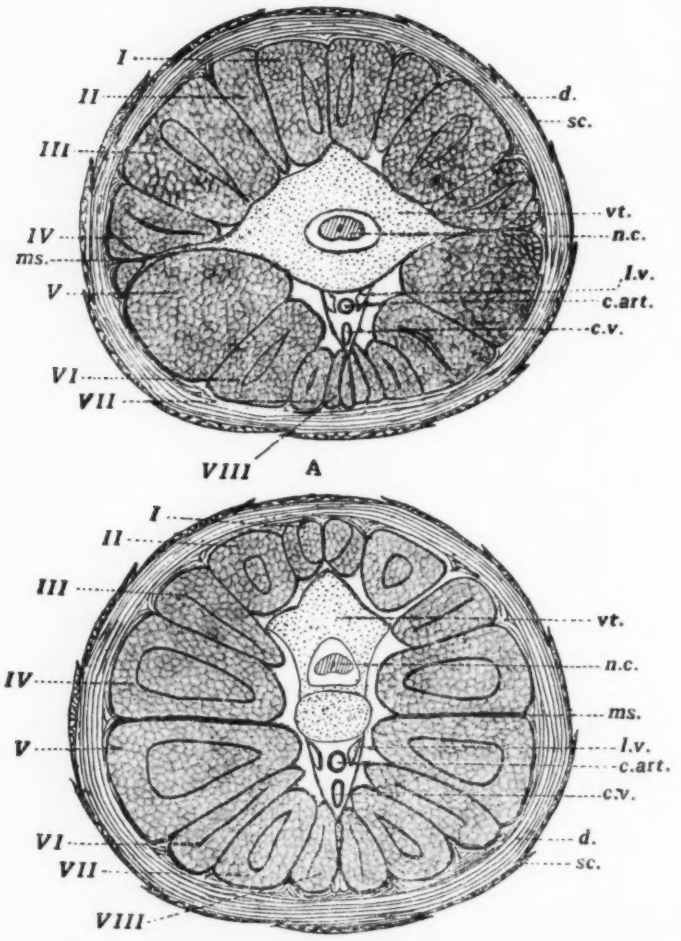


FIG. 4. Transverse sections of the normal tail of *Anguis fragilis*, passing (*A*) through the plane of autotomy and (*B*) through the intervertebral region ( × 18).

*c.art.*, caudal artery ; *c.v.*, caudal vein ; *d.*, dermis ; *l.v.*, lymph vessels ; *m.s.*, mesaxonic septum ; *n.c.*, nerve cord, I–VIII, caudal muscles ; *sc.*, scales ; *vt.*, body of vertebra.

vertebral region (Fig. 4, *B*). First, the neural spines, hæmapophyses and rounded centra are absent in the former, but present in the latter. Secondly, the transverse processes can be made out in the sections passing close to the planes of autotomy, but not elsewhere. Thirdly, the neural space is laterally compressed in the intervertebral region, but dorsoventrally flattened at the autotomy planes. Fourthly, the spinal cord shows a remarkable alteration in its width, being narrower in the intervertebral region than at the autotomy planes.

## IV. The Caudal Musculature

After the skin and the tough sub-cutaneous membrane have been removed, the caudal muscles of the normal tail (Fig. 5) show a myomeric arrangement which is not merely superficial as in *Varanus monitor*, but actual. The author studied the arrangement and dove-tailing of these

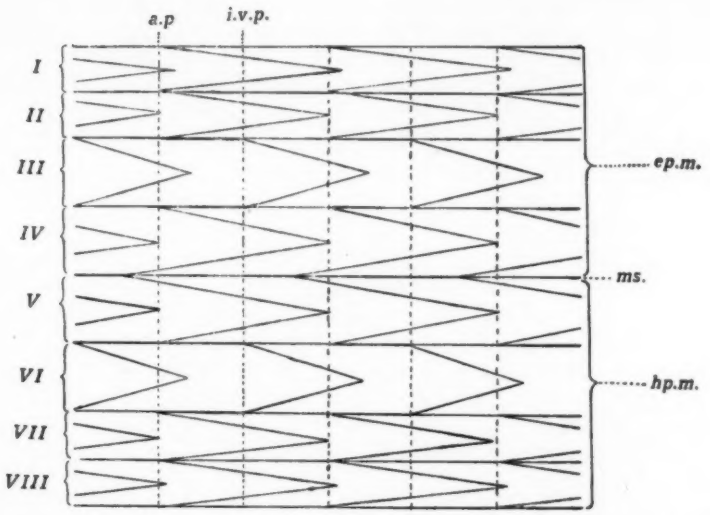


Fig. 5. Schematic diagram showing the disposition of the caudal muscles of *Anguis fragilis*.

*ep.m.*, epiaxonic muscles; *hp.m.*, hypaxonic muscles; *i.v.p.*, inter-vertebral plane; other abbreviations as in previous figures.

muscles both in autotomised surfaces and in serial hand-cut sections. There are four pairs of epiaxonic and four pairs of hypaxonic muscles, and these are arranged and subdivided as in *Hemidactylus flaviviridis* Rüppel. The myomerism corresponds to the autotomic segmentation.

In the regenerated tail, the muscle trunks are not myomerically segmented, but longitudinal. They are more numerous, being in 18 to 20 bundles (Fig. 6).

## V. The Internal Anatomy of the Regenerated Tail

A study of the serial sections (Fig. 6) passing through the regenerated tail of *Anguis fragilis* reveals four interesting features, not hitherto described. First, immediately underneath the epithelium (A) there is a series of irregularly shaped *subdermal scutes*, composed of calcified cartilage and enclosing spaces of varying form. These scutes become much enlarged and occupy

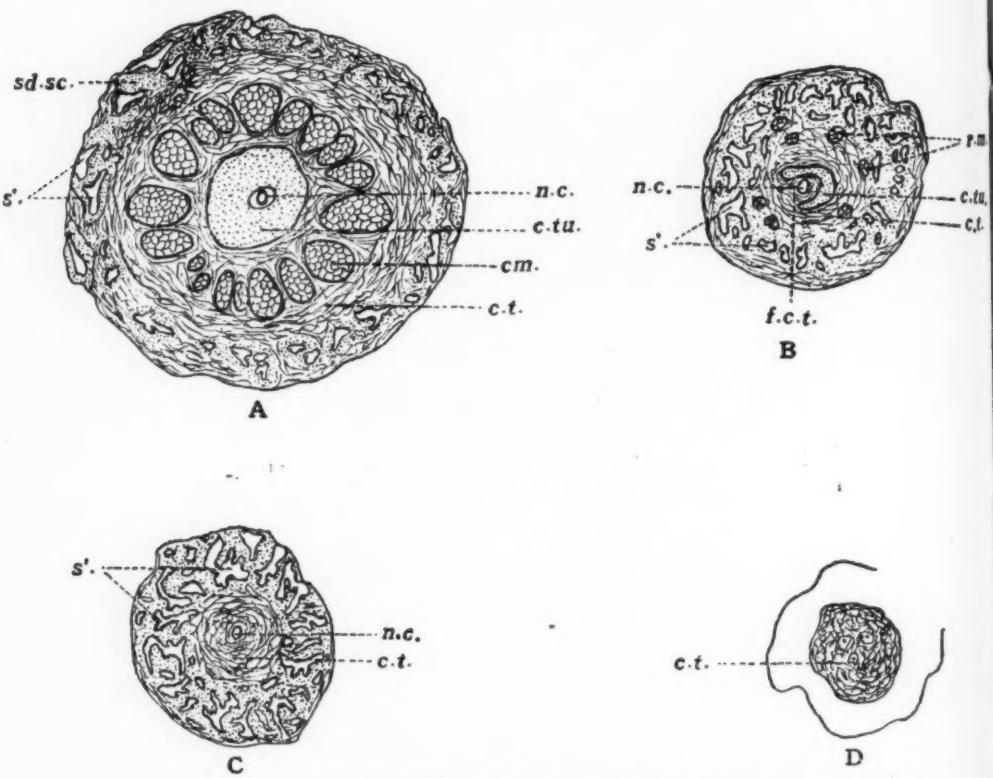


Fig. 6. Transverse sections of the regenerated part of the tail of *Anguis fragilis*: (*A*) a little behind the original tail-stump; (*B*) showing the cartilaginous tube just before its abrupt ending; (*C*) showing the unenclosed nerve cord; and (*D*) passing through the terminal scute (× 24).

*cm.*, regenerated caudal muscle-bundles; *c.t.*, connective tissue; *c.tu.*, regenerated cartilaginous tube; *f.c.t.*, terminal foramen of the cartilaginous tube; *n.c.*, nerve cord; *r.m.*, reduced muscle bands; *s'.*, spaces; *sd.sc.*, sub-dermal scutes.

the major part of the section (B) as we pass backwards. Secondly, in the terminal region (B and C) the cartilaginous tube which replaces the vertebral column, disappears abruptly and thus the nerve cord is left unenclosed. There is a large foramen at the end of cartilaginous tube. Thirdly, the muscle trunks (A-C) disappear and are replaced by a sort of fibrous connective tissue near the end of the tail. Fourthly, the terminal scute (D) has no extension of the musculature, cartilaginous tube and nerve cord.

## VI. Summary

(*a*) The normal tail of *Anguis fragilis* is characterized by the presence of a tough dermal membrane covering the caudal musculature, the opposite dispositions of the median dorsal and median ventral scales, the slender and elongated form of the vertebræ, the flattened zygapophyses, the passing of the plane of cleavage through the transverse processes, the difference in the form of the neural space at the intervertebral and autotomy planes, the narrowing of the spinal cord in the inter-vertebral regions and the presence of the myomerism in the caudal musculature corresponding to the autotomic segmentation.

(*b*) In its regenerated part, it shows the presence of sub-dermal scutes, an abruptly ending cartilaginous tube, the replacement of musculature by fibrous connective tissue, and the formation of a terminal conical scute.

## VII. Acknowledgments

I am extremely grateful to Prof. Beni Charan Mahendra for his supervision and assistance in the present work.

## VIII. Literature Cited

1. Boulengar, G. A. .. "On the Scaling of the Reproduced Tail in Lizards," *Proc. Zool. Soc. London*, 1888, **1**, 351–53.

2. Woodland, W. N. F. .. "Some Observations on Caudal Autotomy and Regeneration in the Gecko (*Hemidactylus flaviviridis* Rüppel) with Notes on the Tails of *Sphenodon* and *Pygopus*," *Quart. Jour. Micr. Sci.*, 1920, **65**, 95–96.

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# ADDITIONS TO FUNGI OF MADRAS—VIII

By T. S. Ramakrishnan, F.A.Sc., and K. Ramakrishnan
(*Mycology Section, Agricultural Research Institute, Coimbatore*)

Received July 2, 1949

(67) *Cystopus molluginicola* Ramakrishnan, T. S. and K., sp. nov.

Sori hypophyllous, circular or irregular, isolated or confluent, raised, white, 0·5–2 mm. diam.; conidiophores cylindric, straight or flexuous, hyaline; conidia globose, subglobose or oblong, without a median annular thickening, hyaline, 15 × 14 $\mu$ (11–20 × 11–16); oospores numerous in the mesophyll of the leaf and the cortex of the peduncle, 52 $\mu$ (42–60) in diam.; epispore dark brown with irregular reticulations.

Soris hypophyllis, rotundis vel irregularibus, sparsis vel confluentibus, inflatulis, albidis, 0·5–2 mm. diam.; conidiophoris cylindricis, rectis vel flexuosis, hyalinis; conidiis globosis, subglobosis vel oblongis, sine incrassatis annulo medio, hyalinis, 15 × 14 $\mu$ (11–20 × 11–16); oosporis numerosis, in foliis et pedunculis hospitis, globosis, 52 $\mu$ (42–60) diam.; episporio fusco brunneo, irregulariter reticulato.

On living leaves and peduncles of *Mollugo nudicaulis* Lam. (Aizoaceæ), Coimbatore, 16–9–1948, T. S. Ramakrishnan.

Fig. 1. *Cystopus molluginicola—a.* conidiophore and conidia; *b.* a chain of conidia; *c.* portion of oospore showing the reticulate markings on the epispore × 275.

The incidence of infection is conspicuous in July-August and November-December. The lowest leaves are the first to be affected. With the development of the oospores the upper surface of the leaf becomes brown in colour and these coloured portions are slightly raised. The oospores can be easily seen as black dots when the leaf is viewed against light. Oospore formation is seen in the peduncle also.

Two species of *Cystopus* have been recorded on plants belonging to the Aizoaceæ, *C. trianthemæ* (Wils.) Sacc. and Trott., on *Trianthema portulacastrum* from Mexico and *C. austro-africanus* (H. and P. Syd.) Sacc. and

Trott., on *Aizoon rigida* from South Africa (Saccardo, 1912, 1926). The fungus under study differs from the former in the absence of the median annular thickening for the conidium and the smaller size of the oospore. It differs from the latter in having irregular reticulations on the episopore of the oospores instead of papillæ.

(68) *Glomerella artocarpi* Delacr.

Saccardo, P. A., *Syll. Fung.*, 1913, **22**, 77.

Syn., *Glœosporium artocarpi* Delacr. in Saccardo, *Syll. Fung.*, 1906, **18**, 454.

On living leaves of *Artocarpus integrifolia* L. (Moraceæ), Thondamuthur (Coimbatore), 10–11–1948, T. S. Ramakrishnan and K. Ramakrishnan.

The conidial stage alone was observed. The fungus produces irregular brown spots on the leaves and causes their premature shedding.

(69) *Mycosphærella agapanthi* Ramakrishnan, T. S. and K., sp. nov.

Spots amphigenous, elongated, grey coloured with reddish margin; perithecia amphigenous, black, globose, wall membranous, dull brown, ostiolate; asci hyaline, subglobose to oblong, wall thickened at the apex, 36 × 21 $\mu$ (25–50 × 14–29); ascospores oblong, 2-celled, hyaline, 19 × 5 $\mu$ (14–25 × 4–7).

Maculis amphigenis, elongatis, canis cum margine rubroso; peritheciis amphigenis, nigris, globosis, ostiolatis, pariete membranaceo, fuscentibus; ascis globosis vel oblongis, hyalinis, pariete apice densato, 36 × 21 $\mu$ (25–50 × 14–29); ascosporis oblongis 2-cellatis, 19 × 5 $\mu$ (14–25 × 4–7).

On living leaves of *Agapanthus umbellatus* L. Heril. (Liliaceæ), Botanical Gardens, Ootacamund, 26–5–1948, T. S. Ramakrishnan and K. Ramakrishnan.

FIG. 2. *Mycosphaerella agapanthi*—asci and ascospores × 275.

The spots commence as small grey drying areas on either side of the leaf. These increase in size and assume a spindle-shaped appearance with a reddish margin. The perithecia are very small and subepidermal but no deep seated.

(70) *Physalospora cordiæ* Ramakrishanan, T. S. and K., sp. nov.

Spots indefinite, amphigenous, irregular, yellowish green, turning brown on drying; perithecia minute, black, crowded in the middle of the spot, but separate, innate, reaching up to the lower epidermis, uniloculate, ostiolate; asci cylindrical with rounded apex, 68 × 15 $\mu$ (43–90 × 7–21), paraphysate, paraphyses coloured orange; ascospores 8, uniseriate, or irregular, hyaline elliptical, 1-celled, 15 × 8 $\mu$ (12–16 × 6–9).

Maculis indefinitis, amphigenis, irregularibus, subflavo viridibus, fusco colore post exsicationem; peritheciis minutis, nigris, medio macularum aggregatis, sed distinctis, innatis, epidermati inferiori attigentibus, uniloculariboss, ostiolatis; ascis cylindricis, apice rotundatis, 68 × 15 $\mu$ (43-90 × 7–21), paraphysatis, paraphysibus aurantiaceis; ascosporis 8, uniseriatis vel irregularibus, hyalinis, ellipticis, unicellularibus, 15 × 8 $\mu$ (12–16 × 6–9).

On living leaves of *Cordia obliqua* Willd. (Boraginaceæ), Walayar, 15–1–1948, T. S. Ramakrishnan and K. Ramakrishnan.

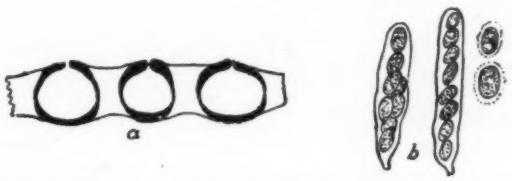


FIG. 3. *Physalospora cordiae—a.* perithecia × 50; *b.* asci and ascospores × 250.

The perithecia appear as black, shining hemispherical protrusions on both sides of the leaf. The wall is thick and dark brown at the top and bottom. The asci gelatinise on maturity and in old perithecia the cavity is filled with the loose ascospores. These are extruded through the ostiole and often collect in waxy, cream coloured masses above the ostiole. The ascospores also have a gelatinous envelope.

*Phyllachora caffra* Syd. has been recorded on *Cordia caffra* from Natal, Africa (Theissen and Sydow, 1915). But the fungus under study is considered different on account of the gelatinisation of the ascus wall and the differences in the measurements of the asci and ascospores.

(71) *Catacauma elettariæ* Ramakrishnan, T. S. and K., sp. nov.

Spots present or not, when present oval, brown with whitish centre, amphigenous; stromata epiphyllous, black, shining, formed in parallel rows, conical with one or rarely more loculi, between the epidermis and palisade; asci cylindric with a small foot, hyaline, 65 × 15 $\mu$ (51–70 ×

12–18); ascospores 8, hyaline, 1-celled, biseriate, oblong, 20 × 7 μ (15–24 × 6–9).

Maculis ovalibus, brunneis, amphigenis; stromata epiphylla, micantia, conica, uni-vel pluriloculata, subepidermia; asci cylindrici, brevipedicellati, hyalini, 65 × 15 μ (51–70 × 12–18); ascosporidia 8, hyalina, unicellata, biseriata, oblonga, 20 × 7 μ (15–24 × 6–9).

On living leaves of *Elettaria cardamomum* Matt. (Zingiberaceæ), Papanasam (Tinnevelly), 15–5–1949, K. Sundaram Pillai.

FIG. 4. *Catacauma elettariae*—*a*. stroma and loculus × 63; *b*. asci × 275.

The fungus is definitely a *Catacauma* as seen from the position of the stroma and the 1-celled hyaline ascospores. The stromata are very minute and appear like black shining dots.

Butler and Bisby (1931) have recorded *Placostroma elettariæ* (B. and Br.) Theiss. and Syd., on cardamom from Bangalore. Theissen and Sydow (1915), who described this fungus on *Elettaria floribunda* from Ceylon based their description of the fungus on Berkeley and Broome's (1875) diagnosis of *Dothidea elettariæ*. The fungus under study agrees with *Placostroma elettariæ* except for the fact that the spores are 1-celled in the former. These spores have usually two vacuoles divided by a central isthmus of protoplasm containing a nucleus. Even when seen with the aid of an oil immersion objective no central septum was seen. It has not been possible to see the type specimen of *Placostroma elettariæ* but the fungus under study cannot belong to this genus as the spores are distinctly 1-celled.

(72) *Phyllachora glycosmidis* Petch.

Petch, T., in *Ann. Roy. Bot. Gard. Peradeniya*, 1917, **6**, p. 3, 227.

Saccardo, P. A., *Syll. Fung.*, 1926, **24**, 602.

On living leaves of *Glycosmis cochinchinensis* Pierre. (Rutaceæ), Burliar (Nilgiris), 22–7–1948, T. S. Ramakrishnan and K. Ramakrishnan.

It has not been possible to see the type specimen but the present fungus agrees with the description of *P. glycosmidis*.

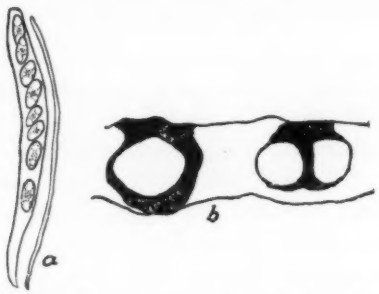


FIG. 5. *Phyllachora glycosmidis—a.* ascus and paraphysis × 410; *b.* perithecia × 60.

(73) *Phyllachora gudalurensis* Ramakrishnan, T. S. and K., sp. nov.

Spots indistinct or light brown in colour, amphigenous; stromata amphigenous, black, shining, multiloculate; asci cylindric-clavate, hyaline 103 × 8 μ (66–126 × 6–9), paraphysate, paraphyses filiform; ascospores 8, uniseriate, oblong or spindle-shaped, hyaline, 1-celled, 11 × 6 μ (10–14 × 4–7).

Maculis indistinctis vel levi brunnei colores, amphigenis; stromata amphigena, atra, micantia, multiloculata; asci cylindrici-clavati, hyalini, 103 × 8 μ (66–126 × 6–9), paraphysati, paraphyses filiformes; ascospridia 8, uniseriata, oblonga vel fusiformia, hyalina, unicellata, 11 × 6 μ (10–14 × 4–7).

FIG. 6. *Phyllachora gudalurensis—a.* stroma and loculi × 40; *b.* ascus × 270; *c.* ascospores × 570.

On living leaves of *Croton oblongifolius* Roxb. (Euphorbiaceæ), Gudalur (Nilgiris), 17–5–1949, T. S. Ramakrishnan.

The spots are clearly visible in some whereas in others there are no definite spots round the stromata. The stromata themselves appear as isolated black spots being uniformly developed on the lower surface with a number of minute projections. On the corresponding upper surface the stromata appear as groups of black shining dots separated by the discoloured leaf tissue.

Theissen and Sydow (1915) have recorded three species of *Phyllachora* on other species of *Croton* from South Africa and South America. But

the fungus under study differs from them in the measurements of the asci and ascospores.

(74) *Phyllachorella micheliæ* Syd.

Sydow, H., *Ann. Myc.*, 1914, **12**, 489.

This fungus was described on *Michelia nilagirica* Zenk. from the Nilgiris (Sydow, 1914). Only perithecia have been recorded so far. In May 1949 an examination of the diseased leaves showed the development of numerous black, subepidermal pycnidia in groups or isolated and closely associated with the perithecia. The pycnidia were ovate and ostiolate. The pycnidiospores were elliptic to oblong, 1-celled, hyaline, and measured $14 \times 7\,\mu$ (9–18 × 6–9). They were extruded in short whitish filamentous masses from the ostioles. The pycnidial stage resembles a *Macrophoma* and appears to represent the imperfect phase of P. *micheliæ* as judged by the close association of the two fungi. These two stages were observed on the leaves of *Michelia champaka* L. also, growing in the Botanical Gardens at Ootacamund.

(75) *Doassansia hygrophilæ* Thirumalachar, in *Lloydia*, 1946, **9**, 24–30.

On living leaves of *Asteracantha longifolia* Nees. (Acanthaceæ), 21-7-1948, Walayar, T. S. Ramakrishnan and K. Ramakrishnan.

(76) *Entyloma fuscum* Schroet.

Clinton, G. P., "North American Ustilaginæ," *Proc. Bost. Nat. Hist. Soc.*, 1904, **31**, 471.

Mundkur, B. B., *Trans. Brit. Mycol. Soc.*, 1940, **24**, 331.

On living leaves of *Papaver* sp. (Garden variety) (Papaveraceæ), Ootacamund, 8–10–1948, T. S. Ramakrishnan.

This smut was observed on the leaves of the garden poppy at the Botanical Gardens, Ootacamund. The spots are amphigenous, brown, circular or irregular. They are covered by white growths on the lower surface. Hyaline filiform, falcate, 1-celled conidia, measuring $10–14 \times 1{\cdot}5–2{\cdot}5\,\mu$, are formed on the spots.

(77) *Melampsora salicis-albæ* Klebh.

Klebhan, H., in *Zeits. Pflanzenkrankh.*, 1902, **12**, 21–22.

Syn. *Melampsora allii-salicis-albæ* Klebh. Sacc., *Syll. Fung.*, **17**, 266.

On living leaves and twigs of *Salix babylonica* L. (Salicaceæ), Botanical Gardens, Ootacamund, 28–5–1948, T. S. Ramakrishnan; on living leaves

and petioles of *Salix tetrasperma* Roxb. Perur (Coimbatore), 1-1-1949, T. S. Ramakrishnan and K. Ramakrishnan.

The uredial stage alone was present on the two hosts as bright orange coloured sori. The identification is tentative as the other stages have not been seen.

(78) *Urediniopsis macrosperma* (Cke.) Magn.

Arthur, J. C., *North American Flora*, 1925, **7** (10), 684–87.

Arthur, J. C., *Manual of the Rusts in United States and Canada*, p. 5, 1934.

Syn. *Urediniopsis pteridis* (Diet. and Holw.) Arthur, in *North American Flora*, 1907, **7**, 116.

*Milesina pteridis* (Diet. and Holw.) Syd. in *Monogr. Ured.*, 1915, **3**, 481.

On living leaves of *Pteridium aquilinum* (L.) Kuhn., Anamalais, 30-1-1949, D. Marudarajan and T. S. Ramakrishnan.

FIG. 7. *Urediniopsis macrosperma*—urediospores × 425.

This rust has undergone several changes in nomenclature. In the early description of *Urediniopsis pteridis*, Arthur (1907) has given the description of the uredia as follows:—" urediospores oval or fusiform, 11–18 × 30–58 $\mu$, apex acute, rarely tipped with a short stout beak, wall thin with a few scattered papilla, beside the two sharply defined longitudinal rows of loosely set papilla. In 1925 Arthur reduced *Urediniopsis pteridis* to a synonym of *U. macrosperma*, and in the amended description he states, " the urediospores are obovate or broadly ellipsoid, more or less angular, 14–26 × 26–45$\mu$, wall closely and rather finely verrucose". A further revision of the description made by him in 1934 (Arthur, 1934) agrees closely with the original description (Arthur, 1907).

On the Anamalais and the Nilgiris two rusts are apparently present on this host, one of which is now identified as *U. macrosperma*. This has whitish hypophyllous uredia covered by delicate peridium. The spores are pushed out through a central opening. They are fusiform or oval with an acute apex often tipped with a pointed beak 4–10 $\mu$ long. The spores measure 40 × 14 $\mu$ (30–51 × 12–17). The wall is colourless and rather closely verrucose. One longitudinal row of close set papillæ is evident,

Teliospores which are rare are seen in the mesophyll cells as delicate, globoid, 1–3 celled hyaline smooth walled structures. This rust agrees with the latest description of *U. macrosperma.*

(79) *Puccinia coronata* Corda.

Sydow, H. and P., *Monogr. Ured.*, 1, 320.

Butler, E. J. and Bisby, G. R., *Fungi of India*, p. 66, 1931.

Ramakrishnan, T. S. and K., *Proc. Ind. Acad. Sci.*, 1948, **28**, 65.

The æcial stage of this rust was seen on the leaves of *Rhamnus virgatus* Roxb. (Rhamnaceæ) at Nanjanad, Nilgiris, 28–5–1948, T. S. Ramakrishnan and K. Ramakrishnan.

(80) *Uromyces nilagiricus* Ramakrishnan, T. S. and K., sp. nov.

Rust spot hypertrophied, up to 7 mm. diam.; pycnia amphigenous, subepidermal, oval, 240 × 210 $\mu$; æcia amphigenous, deeply sunk, cupulate, peridium of one layer of polygonal, colourless, thick-walled, verrucose cells; æciospores angular, globose, catenulate, yellowish orange in colour, wall unevenly thickened, highly verrucose, 30 × 25 $\mu$ (24–33 × 18–30); uredia wanting; telia amphigenous, chocolate brown, subepidermal, erumpent; teliospores 1-celled, elliptic to rhomboid, narrow towards the base, 36 × 26 $\mu$ (30–45 × 21–30) apex rounded, thickened up to 8 $\mu$, orange brown in colour, wall smooth, pedicellate, pedicel hyaline, up to 130 $\mu$ long.

Maculis amplificatis, usque 7 mm. diam.; pycnia amphigena, subepidermia, ovalia, 240 × 210 $\mu$; æcia amphigena, profunde immersa, cupulata, cellulis peridiis polygonis, hyalinis, incrassatis, verrucosis; æciosporis angulato globosis, catenulatis luteo colore, episporio inequaliter incrassato, prominenter verrucoso 30 × 25 $\mu$ (24–33 × 18–30); uredia ignota; telia amphigena, subepidermia, erumpentia, brunnea; teliosporis unicellularibus, ellipticis vel rhomboidis, basi attenuatis, 36 × 28 $\mu$ (30–45 × 21–30) apice rotundato, incrassato usque 8 $\mu$, episporio levi, aurantei brunnei colores, pedicellatis, pedicellis hyalinis, usque 130 $\mu$ longis.

On living leaves of *Loranthus* sp. (Loranthaceæ) parasitic on *Citrus reticulata*, Kotagiri, 25–4–1949, D. Marudarajan.

The pycnia and æcia occur on swollen concavo-convex areas. The convexity may be towards either surface. The æcia project as short white columns with lacerated and recurved margins. Telia are not on the same spots as æcia but occur on the same leaf.

Cummins (1931) mentions the occurrence of seven species of *Uromyces* on hosts belonging to the Loranthaceæ. But in all of them the wall of the

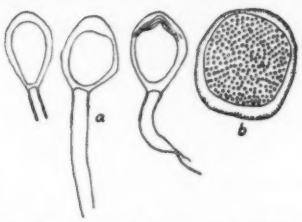


FIG. 8. *Uromyces nilagiricus—a.* Teliospores × 270; *b.* aeciospore × 570.

teliospore is either ridged or reticulate and never smooth. In the rust under study the wall of the teliospore is completely smooth and no markings are visible even under the oil immersion objective. It is therefore considered different from those recorded so far.

(81) *Kordyana indica* Gaumn.

Syn. *K. tradescantiæ* (Pat.) Racib. Saccardo, P. A., *Syll. Fung.*, 1902, **16**, 199. Gäumann, E., *Ann. Mycol.*, 1922, **20**, 264. Butler, E. J. and Bisby, G. R., *Fungi of India*, 1931, p. 103.

On living leaves of *Commelina bengalensis* L. (Commelinaceæ), Walayar, 28-8-1948, T. S. Ramakrishnan and K. Ramakrishnan.

Bright yellowish spots are seen on the upper surface of the leaves. Corresponding to these, on the lower surface longitudinal rows of minute white groups of basidia are evident.

(82) *Cercospora careyæ* Ramakrishnan, T. S. and K., sp. nov.

Spots amphigenous, isolated, irregular, brown; hyphæ internal; conidiophores amphigenous, emerging through stomata, straight or bent, up to 4-septate, 70 × 4 $\mu$ (57–99 × 3–4·5), brown, apex geniculate; conidia obclavate, straight or curved, 1–4 septate, subhyaline to light brown, 45 × 5 $\mu$ (15–84 × 3–6).

Maculis amphigenis isolatis, irregularibus, brunneis; hyphis interioribus; conidiophoris amphigenis, ex stomatibus erumpentibus, rectis vel curvatis usque 4-septatis, brunneis, 70 × 4 $\mu$ (57–99 × 3–4·5), apice geniculatis; conidiis obclavatis, rectis vel curvatis 1–4 septatis, subhyalinis vel pallide brunneis, 45 × 5 $\mu$ (15–84 × 3–6).

On living leaves of *Careya arborea* Roxb. (Lecythidaceæ), Gudalur, 17-5-1949, T. S. Ramakrishnan.

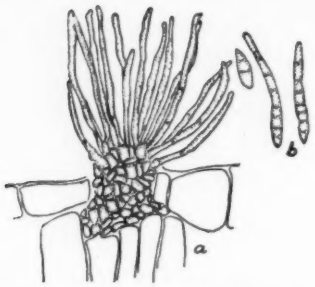


FIG. 9. *Cercospora careyae—a.* a cluster of conidiophores; *b.* Conidia × 275.

A small, multicellular stroma is formed in the substomatal space. The stroma projects outside later. After the formation of the conidiophores and conidia the spot presents a smoky brown appearance.

(83) *Cercospora dalbergicola* Ramakrishnan, T. S. and K., sp. nov.

Spots minute, amphigenous, prominent on the lower surface; conidiophores produced in fascicles on small stromata, hypophyllous, fascicles isolated or confluent, septate, sometimes branched, with geniculate apices, 82–123 × 4–7 $\mu$, light brown; conidia usually one-septate, sometimes 2-septate, obclavate, subhyaline, or light brown, 29 × 6 $\mu$ (22·37 × 4–8).

Maculis minutis, amphigenis, inferiori superfecie prominentibus; conidiophoris fasciculatis, in parvis stromatibus, fasciculis isolatis vel confluentis, septatis, aliquando ramificatis, cum apicibus geniculatis, fuscatis, 82–123 × 4–7 $\mu$; conidiis habitualiter 1-septatis, aliquando 2-septatis, obclavatis, subhyalinis vel fuscatis, 29 × 6 $\mu$ (22–37 × 4–8).

On living leaves of *Dalbergia volubilis* Roxb. (Papilionaceæ), Walayar, 28–7–1948, T. S. Ramakrishnan and K. Ramakrishnan.

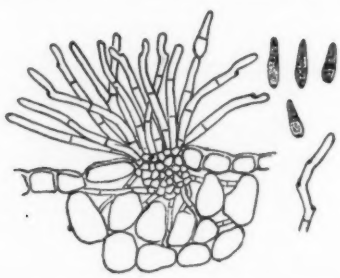

FIG. 10. *Cercospora dalbergicola* conidiophores and conidia × 250.

Greyish brown raised spots are formed on the lower surface. One or more clusters of conidiophores are formed on these and they give a powdery appearance to the growth. *Cercospora sissoo* Syd. has been recorded on *Dalbergia sissoo* from Allahabad, India (Sydow and Mitter, 1933). The fungus under study differs from this in the appearance and measurements of the conidiophores and conidia.

(84) *Cercospora eucalypti* Cke. and Mass.

Saccardo, P. A., *Syll. Fung.*, 1892, **10**, 644.

On living leaves of *Eucalyptus ficifolia* (Myrtaceæ), Ootacamund, 8–7–1948, T. S. Ramakrishnan.

The fungus causes circular to irregular spots with reddish margins on the leaves.

(85) *Cercospora sapindi-emarginati* Ramakrishnan, T. S. and K. sp. nov.

Spots amphigenous, irregular, 2–3 mm. across, yellowish, studded with black dots; conidiophores developed on subepidermal erumpent stromata, amphigenous, bursting through the epidermis, brown, continuous; conidia long-obclavate, many celled, olive brown, 48 × 5 $\mu$ (22–105 × 4–7).

Maculis amphigenis, irregularibus, 2–3 mm. diam. flavescentibus, nigris punctatis conspersis; conidiophoris ex subepidermalibus erumpentibus, stromatibus surgentibus, amphigenis, ex epidermate erumpentibus, fuscis, continuis; conidiis, longiobclavatis, pluricellularibus, olivaceo fuscis, 48 × 5 $\mu$ (22–105 × 4–7).

On living leaves of *Sapindus emarginatus* Vahl. (Sapindaceæ), Anakapalle, 27–2–1948, T. S. Ramakrishnan.

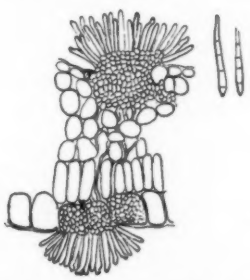

FIG. 11. *Cercospora sapindi-emarginati* conidiophores and conidia × 130.

The spots are often indistinct, but sometimes become whitish. The epidermis covering the stroma is raised like a flap. Portions of the stroma

fill the epidermal cells forming pale brown plectenchymatous growths. Each spot may bear one or more stromata which appear as black dots.

(86) *Cercospora tylophoræ* Ramakrishnan, T. S. and K., sp. nov.

Spots indistinct, fruiting amphigenous, maroon coloured; conidiophores in fascicles, on the upper surface coming out through the stoma, on the lower surface diffuse from superficial mycelium, septate; conidia cylindric to obclavate, 3–8 celled, 67 × 5 $\mu$ (29–122 × 4–7), straight, subhyaline to light brown.

Maculis indistinctis, amphigene fructificantibus, fusco coccineis, rubidulis; conidiophoris in superiori superficie fasciculatis ex stomatibus exeuntibus, diffuso superficiali mycelio in superficei inferiori, septatis; conidiis cylindricoobclavatis, 3–8 cellulatis, erectis, subhyalinis vel fuscatis, 67 × 5 $\mu$ (29–122 × 4–7).

On living leaves of *Tylophora asthmatica* W. and A. (Asclepiadaceæ), Walayar, 28–8–1948, T. S. Ramakrishnan and K. Ramakrishnan.

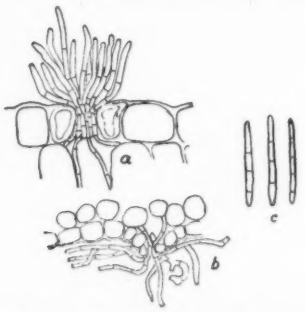


FIG. 12. *a*. Section of leaf showing upper surface; *b*. lower surface; *c*. conidia × 130.

The fungus does not produce any distinct spots. The growth is more prominent on the lower surface as maroon coloured irregular patches. On the upper surface more compact maroon coloured dot-like outgrowths are seen. The internal mycelium produces compact fascicles of conidiophores which emerge through the stomata on the upper surface. On the lower surface, however, the hyphæ come out through the stoma and form a loose growth outside the leaf. From this, conidiophores which are not usually in fascicles are given off at different places.

(87) *Glæosporium zonatum* Ramakrishnan, T. S. and K., sp. nov.

Spots extensive, distinctly zonate, amphigenous, grey to brown; acervuli numerous, amphigenous, but mostly epiphyllous, intraepidermal; conidio-

phores nonseptate, hyaline; conidia cylindric or oblong, straight or curved, 1 celled, hyaline, with rounded ends, 17 × 5 μ (12–21 × 3–6).

Maculæ amphigenæ, zonatæ, brunneæ; acervulis numerosis, amphigenis, sed prominenter epiphyllis, intraepidermalibus; conidiophoris nonseptatis, hyalinis; conidiis cylindricis vel oblongis, rectis vel curvatis, unicellatis, hyalinis, apice rotundatis, 17 × 5 μ (12–21 × 3–6).

FIG. 13. *Gloeosporium zonatum*—conidia × 570.

On living leaves of *Cœlogyne* sp. (Orchidaceæ), Ambillimalai estates, Gudalur (Nilgiris), 16–5–1949, T. S. Ramakrishnan.

The spots extend over a large portion of the leaf and exhibit a number of successive zones limited by wavy dark lines. The acervuli appear as minute dots and are arranged in concentric circles. The development of the acervulus is mostly within the epidermal cells. The outer walls of the epidermal cells burst open and the spores are exposed. The stroma is dark brown. The spores on germination develop the characteristic appressoria.

(88) *Septoglœum scutiæ* Ramakrishnan, T. S. and K., sp. nov.

Spots minute, amphigenous, dark brown, with a dark raised rim, irregular; acervuli epiphyllous, subepidermal; conidiophores clavate, nonseptate; conidia straight or curved, cylindric to clavate, 1–3 septate, subhyaline, 44 × 2 μ (22–73 × 1·4–2·8).

Maculæ parvæ, amphigenæ, fusci brunnei colores, irregulares; acervulis epiphyllis, subepidermalibus; conidiophoris clavatis, nonseptatis; conidiis rectis vel curvatis, cylindircis vel clavatis, 1–3 septatis, subhyalinis, 44 × 2 μ (22–73 × 1·4–2·8).

On living leaves of *Scutia myrtina* Kurz. (Rhamnaceæ), Kotagiri, 25–4–1949, T. S. Ramakrishnan.

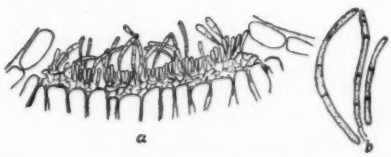


FIG. 14. *Septogloeum scutiae*—*a*. acervulus × 205; *b*. conidia × 425.

The spots are small usually 2 × 1 mm. and appear to be thickened owing to the raised dark margin. Two to four acervuli are formed in each spot. These are reddish brown in colour and remain covered by the epidermis for a long time.

(89) *Discella cedrelæ* Ramakrishnan, T. S. and K., sp. nov.

Spots amphigenous, circular or irregular, with shining black, irregular, flattened or discoid pycnidia arranged around a central straw coloured portion, 2–4 mm. diam.; pycnidia amphigenous, subepidermal, opening irregularly; pycnidiospores pedicellate, oblong, 2-celled, lower cell smaller, subhyaline, smooth, 18 × 9 μ (13–20 × 7–12).

Maculis amphigenis, orbicularibus vel irregularibus, cum pycnidiis fulgenter nigris irregularibus applanatis vel discoideis plus minus in circulism circa centralem stramineam partem dispositis, 2–4 mm. diam.; pycnidiis amphigenis, sub-epidermalibus, irregulariter aperientibus; pycnidiosporis pedicellatis, oblongis, 2-cellularis, ima cellula parviori, subhyalinis, lævibus, 18×9 μ (13–20×7–12).

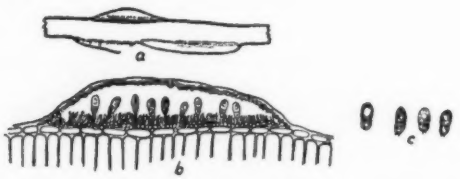


FIG. 15. *Discella cedrelae—a.* section of pycnidia (diagrammatic); *b.* section of a pycndium × 250; *c.* pycnidiospores × 250.

On living leaves of *Cedrela toona* Roxb. (Meliaceæ), Sim's Park, Coonoor, 9–10–1947, T. S. Ramakrishnan.

The spots are many on a leaf. The pycnidia occur like black incrustations irregularly arranged around a central clear portion or as irregular black crusts without a central space. They are very much flattened. The sporiferous portion is arched over by a layer of fungal tissue made up of dark cells. This roof ruptures irregularly and the spores are exposed.

(90) *Phleospora cassiæ* Ramakrishnan, T. S. and K., sp. nov.

Spots amphigenous, circular or irregular, brown to tawney olive, lower surface zonate, buffy brown in parts, 5–35 mm. diam.; pycnidia arranged in concentric rings, incomplete, hypophyllous, subepidermal, with a wide mouth, immersed to half the depth of the leaf; pycnidiospores narrow,

elongated, slightly curved, borne on closely arranged stalks, normally 3-septate, 32 × 4 $\mu$ (22–41 × 2–5).

Maculis amphigenis, circularibus, vel irregularibus, fuscoflavo olivaceis, inferiori superficei zonata, luteo fuscis partialiter, 5–35 mm. diam.; pycnidiis concentricis, circulis dispositi hospitis, hypophyllis, subepidermalibus, incompletis, cum lato ostio, usque dimidia partem foliorum immersis; pycnidiosporis angustis, acicularibus, leviter curvatis, dense collocatis pedicellis gestatis, 3-septatis, hyalinis, 32 × 4 $\mu$ (22–41 × 2–5).

On living leaves of *Cassia fistula* L. (Cæsalpiniaceæ) on the road from Kallar to Burliar (Nilgiris), 10–11–1946, T. S. Ramakrishnan.

FIG. 16. *Phleospora cassiae*—pycnidiospores × 530.

The spots are found either in the middle or along the margin of the leaflets and may sometimes involve the major portion of the leaflet by coalescence. The lower surface is dotted with concentric rings of small pycnidia. When fresh, gelatinous pinkish masses of spores project through the wide openings of the pycnidia. The affected portions of the leaf dry up quickly and become brittle.

(91) *Septoria graminum* Desm.

Saccardo, P. A., *Syll. Fung.*, 1884, **3**, 565.

On living leaves of *Digitaria marginata* Link. (Gramineæ), Walayar, 12–11–1948, T. S. Ramakrishnan.

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## REFERENCES


Berkeley, J. M. and Broome, C. E. .. *Jour. Linn. Soc.*, 1875, **14**, 134.

Cummins, G. B. .. *Mycologia*, 1931, **31**, 173–74.

Saccardo, P. A. .. *Syll. Fung.*, 1912, **21**, 858.

——————— .. *Ibid.*, 1926, **24**, 33.

Sydow, H. .. *Ann. Mycol.*, 1914, **12**, 489.

——— and Mitter, J. H. .. *Ibid.*, 1933, **33**, 84–97.

Theissen, F., and Sydow, H. .. *Ibid.*, 1915, **13**, 522–24, 548.

# SOME FACTORS GOVERNING THE VITAMIN C CONTENT OF *TRIGONELLA FOENUM-GRAECUM**

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There is considerable controversy regarding the influence of various factors governing the formation of vitamin C in plants. The observations of numerous investigators indicate that variations in the vitamin C content of a plant are related to many factors, including varietal differences, influence of light and variations in climate and seasonal differences and to a lesser extent fertilizer practices. Work reported from this country on this important subject, especially with reference to vegetable crops, is rather scanty. An attempt was, therefore, made to study the effect of certain factors on the vitamin C content of some Indian vegetables. To begin with, *Trigonella Foenum-græcum*, a leafy vegetable of some importance, was chosen because of the lack of any specific information on the various factors governing the formation of vitamin C in this leafy vegetable, coupled with the quick growth of the crop and the easiness of cultivation on a small scale and in pot cultures throughout the year. The localization of the vitamin in different parts of the plant, the vitamin content at various stages of plant growth, the effect of light, and manganese, nitrogen and potassium fertilization were studied in some detail. The results obtained during the course of these studies are presented in the following pages.

## Materials and Methods

*Materials.*—A single strain of *Trigonella Foenum-græcum* was used in these experiments. The plant materials used for the estimation of vitamin C were from those grown in the laboratory garden and glass-house in either earthenware pots containing equal amounts of garden soil or in glazed pots filled with acid-washed, neutral sand supplied with known nutrient solutions. The details regarding these are given under appropriate heads when the different experiments are dealt with separately.

*Method of Estimating Vitamin C.*—Tillman's 2:6-dichlorophenol-indophenol method as modified by Harris and Olliver (1942) was employed in

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the estimation of the vitamin C content. Metaphosphoric acid (5%) was used as the extracting agent. Since tests for the presence of dehydroascorbic acid were negative, the treatment of the extracts with $H_2S$ was omitted in all the estimations.

When more than one sample had to be analysed, the plant material was harvested as stated under the different experiments, and weighed out immediately and kept immersed in the extracting agent in Pyrex glass beakers, which in turn were stored in a refrigerator at 10° C. The extraction and titration were carried out as rapidly as possible. Whenever the various components of the plant had to be tested, the leaves were extracted and titrated first, the flowers next, the stem and the roots last.

## Experimental

1. *Localization of Vitamin C in the Plant.*

To determine the vitamin content in the different parts of the plant, 20 plants of uniform growth were chosen from the seedlings raised in earthenware pots containing garden soil. They were washed free of soil and dried in the folds of a clean, dry towel. The different plant parts, *viz.*, the leaf-blades, petioles, cotyledons, stems, young leaf buds, flower buds, blossomed flowers and roots were separated and weighed to 0·01 g. and were immediately kept covered with metaphosphoric acid extracting agent. The following table presents the vitamin C content of the various parts.

Table I

*Showing the vitamin C content in different parts of the plant* (20 *seedlings at the time of flowering*)

| Plant parts | Fresh wt. in g. | Vitamin C content in mg. | Vitamin C mg./100 g. |
|---|---|---|---|
| Leaf-blades .. | 3·22 | 1·923 | 59·72 |
| Petioles .. | 0·83 | 0·102 | 12·05 |
| Buds .. | 1·02 | 0·417 | 40·89 |
| Cotyledons .. | 1·14 | 0·160 | 14·03 |
| Stem .. | 1·75 | 0·162 | 9·23 |
| Flower buds .. | 0·37 | 0·107 | 28·92 |
| Flowers .. | 0·40 | 0·143 | 35·75 |
| Roots .. | 1·08 | 0·083 | 7·68 |

The bulk of the vitamin C content was found in the leaves. Even in the leaves, the leaf-blade contained more of the vitamin than the petiole. Next to the leaf the flowers contained an appreciable amount of the vitamin. The stem and the roots were seen to have only a small fraction of the total vitamin C content of the plant. Therefore, in the work that follows the shoot alone was used for the vitamin C estimations.

2. *Vitamin C Content at Different Stages of Plant Growth.*

The vegetable material bought from a local market showed a vitamin content of 39·1 mg. per 100 g. of the fresh material. It was also observed that the material obtained from the market hardly reached the second leaf stage. It was, therefore, thought desirable to ascertain the optimum period when the plant could be harvested for the maximum vitamin content.

Seeds of this vegetable were sown in 24 earthenware pots of uniform size containing weighed amount of the same compost soil and watered with measured amount of tap water maintaining approximately the same moisture level in all the pots; six sets of seeds were sown at weekly intervals, 4 pots each week. The seedlings were harvested at the same time six weeks after the first set was sown, thus the first sown set was six weeks old, while the last sown was one week old. The plants were cut at the cotyledonary node so as to ensure uniformity. Twenty seedlings were chosen at random from the four pots per age group, weighed and immediately placed in a beaker containing the extracting agent. The vitamin values of these samples are presented in the following table.

TABLE II

*Showing the vitamin C content at different stages of growth*

| Age of plants in weeks | Fresh wt. in g. (20 seedlings) | Vitamin C content in mg. | Vitamin C mg./100 g. |
|---|---|---|---|
| 1 | 2·20 | 0·74 | 33·6 |
| 2 | 3·16 | 1·29 | 40·8 |
| 3 | 3·41 | 1·66 | 48·6 |
| 4 | 4·10 | 2·31 | 56·2 |
| 5 | 6·14 | 3·57 | 58·1 |
| 6 | 7·49 | 3·23 | 43·1 |

The vitamin C content increased stage by stage till the fifth week and decreased in the sixth week, when the plants began to flower. No appre-

ciable difference in the vitamin content in the fourth and fifth week samplings was noticeable and it, therefore, appeared that for all practical purposes the vegetable could be harvested by about the fourth week.

3. *Effect of Light on the Vitamin C Content.*

Three experiments were carried out to determine how far light affected the vitamin C content of this vegetable. The first was to determine the vitamin content at different periods of the day, the second was to find out if there was any difference in the vitamin content of the plants grown in the open garden and those grown in a partly shaded glass-house, and the third to have an idea of the effect of darkness and light on the vitamin content in *Trigonella* seedlings.

(*a*) *Vitamin C content at different times of the day.*—The seedlings were grown in direct sunshine in earthenware pots and were sampled (cut at the cotyledonary node) for the vitamin estimation at the fourth week stage. Two representative 4 g. samples were taken at two-hourly intervals, one for the estimation of the vitamin content and the other for the dry weight determination. The first test was made at 8 A.M. and then afterwards at two-hourly intervals till 4 P.M., the last estimation being done at 7 P.M., and this alone at a three-hourly interval. The results obtained are summarised in Table III.

TABLE III

*Showing the vitamin C content of* Trigonella *at different times of the day*

| Time | Vitamin C on fresh weight basis mg./100 g. | Vitamin C on dry weight basis mg./100 g. |
|---|---|---|
| 8 a.m. | 44·5 | 249·3 |
| 10 a.m. | 49·0 | 280·8 |
| 12 noon | 56·7 | 314·4 |
| 2 p.m. | 55·5 | 309·2 |
| 4 p.m. | 51·0 | 291·0 |
| 7 p.m. | 45·5 | 262·3 |

The vitamin C content of the shoot was seen to increase from 44·5 mg. per 100 g. of fresh material at 8 A.M. to 56·7 mg. per 100 g. at 12 noon, after which time it gradually decreased to 45·5 mg. per 100 g. at 7 P.M. On a

dry-weight basis also the vitamin content was observed to show an increase till 12 noon.

(*b*) *Vitamin C content of the plants grown outside and inside the glass-house.*—Seedlings exposed to direct sunlight as against those grown in diffused light were harvested when four weeks old, and two representative 4 g. samples were weighed out from each of these two groups. The dry weight was determined from one lot and the vitamin content from the other. The results are as follows:

TABLE IV

*Showing the vitamin C content of the plants grown outside and inside the glass-house*

| Material | Vitamin C on fresh weight basis mg./100 g. | Vitamin C on dry weight basis mg./100 g. |
|---|---|---|
| Plants grown outside .. | 61·0 | 335·2 |
| Plants grown in glass-house .. | 49·75 | 291·3 |

The plants grown outside in the garden showed a higher vitamin content both on fresh and dry-weight basis than those grown in the glass-house under diffused light conditions.

(*c*) *Effect of light and darkness.*—Out of the pots sown with *Trigonella* and kept out in the open garden for the above experiment, six were retained for this experiment. When the plants were four weeks old, two pots were kept in a dark chamber at 4 P.M. to be retained there for 24 hours, *i.e.*, till 4 P.M. on the following day, and yet another two were kept in the dark chamber

TABLE V

*Showing the vitamin C content of the seedlings as affected by darkness and light*

| Treatments | Vitamin C on fresh wt. basis mg./100 g. | Vitamin C on dry wt. basis mg./100 g. |
|---|---|---|
| Plants grown in sunlight for 28 days .. | 52·0 | 293·7 |
| „ followed by 24 hours in darkness .. | 39·5 | 228·3 |
| „ followed by 8 hours in daylight .. | 46·3 | 262·7 |

for 24 hours from 8 A.M. to 8 A.M. on the following day, after which period these last two were removed to the field for eight hours till 4 P.M., when plants from all these three sets of pots were harvested for the estimation of the vitamin content. The results obtained are presented in Table V.

The plants grown in the garden under natural conditions contained a high vitamin content. Those kept for 24 hours in complete darkness showed a decrease in the vitamin value, while those kept in darkness for the same period but later on exposed to day-light for eight hours had shown an increase over that in the darkness, but still less than those that remained in the open throughout.

4. *Effect of Manganese on the Vitamin C Content of* Trigonella Foenum-græcum.

To study the effect of varying amounts of added manganese on the vitamin C content of the plants grown in a garden soil containing a small amount of organic matter, 34 glazed earthenware jars of uniform size (7″ × 5″) containing equal amounts of a well mixed garden soil (3,000 g.) were chosen. Four pots were allotted for each treatment, the number of treatments including the control being eight. Two pots were left out for ascertaining the loss in moisture level. The seven treatments were the addition of manganese sulphate ($MnSO_4.4H_2O$) in varying concentrations ranging from 0·05 to 2·0 g. per pot. The salt was added to the soil in the form

TABLE VI

*Showing the effect of the addition of manganese sulphate to the soil on the fresh weight, dry weight and vitamin C content of the seedlings*

| Treatments | Fresh weight in g. (10 seedlings) | Dry weight in g. | Vitamin C content mg./100 g. of fresh material |
|---|---|---|---|
| A Control—untreated | 2·01 | 0·360 | 48·3 |
| B 0·05 g. $MnSO_4.4H_2O$ | 2·45 | 0·516 | 56·4 |
| C 0·10 ,, | 2·26 | 0·472 | 54·6 |
| D 0·25 ,, | 2·16 | 0·429 | 51·1 |
| E 0·50 ,, | 2·21 | 0·462 | 50·6 |
| F 0·75 ,, | 2·08 | 0·379 | 48·5 |
| G 1·00 ,, | 2·06 | 0·379 | 48·9 |
| H 2·00 ,, | 1·92 | 0·355 | 48·5 |
| Critical difference P = 0·05 | 0·09 | 0·009 | 1·6 |
| Conclusion .. | B C E D F G A H<br>or<br>B > C = E ; E = D ; D = F ; F = G = A ; A = H | B C E D F G A H<br>or<br>B > C > E > D > F = G > A = H | B C D E G H F A<br>or<br>C > D = E > G = B H = F = A |

of a dilute solution, and 40 seeds were sown in each pot. The seedlings were thinned to 30 on germination. These pots were watered with tap water at two-day intervals maintaining the moisture level.

The 32 pots were kept in a glass-house in the form of four blocks, the pots representing the different treatments in each being arranged in a randomized manner. The seedlings were harvested (cut at 2 cm. below the cotyledonary node) when they were four weeks old, and from each pot two lots of ten seedlings each were separated, one for the fresh and dry weight determination, and the other for the vitamin C estimation. The results were statistically computed and are presented in Table VI.

A significant increase in the fresh weight, dry weight and vitamin C content of the seedlings was obtained by the addition of 0·05 g. of $MnSO_4$ to the soil. Higher doses of $MnSO_4$ did not give a significant increase in the vitamin C content of the seedlings.

5. *The Effect of Nitrogen and Potassium Fertilization on the Vitamin C Content.*

To study the effect of nitrogen and potassium fertilization on the vitamin C content of this vegetable, sand cultures were resorted to as it was only with such controlled cultures that the effect of varying the amounts of the particular nutrient element without altering the quantity of the other essential elements could be studied with certainty. The sand chosen was river sand locally obtained, acid-washed and neutral, with a loss on ignition of 0·23%.

A nutrient solution as was used by Woodman (1939) was chosen, as it could be easily prepared and used with advantage especially when it was desired to alter the amount of one particular nutrient element without changing the composition of the other elements. Preliminary experiments with such a nutrient solution showed that this vegetable could be grown in sand cultures supplied with a nutrient solution containing the following pure chemicals (analytical reagents): sodium nitrate, potassium sulphate, calcium hydrogen phosphate, disodium hydrogen phosphate, magnesium sulphate, ferrous sulphate, boric acid, and sulphates of manganese, zinc and copper, giving the composition noted below:

| *N* | *P* | *K* | *Ca* | *Mg* | *Fe* | *S* | *Mn* | *B* | *Zn* | *Cu* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 33·6 | 21·7 | 23·5 | 8·0 | 4·9 | 1·1 | 16·6 | 0·18 | 0·07 | 0·07 | 0·02 | p.p.m. |

(the small amount of the sulphates from the trace elements was not taken into consideration). The increase of sulphur in the nutrient medium upto 30 p.p.m. did not affect the normal growth of the plant and, therefore, no special attention was paid to maintain the exact amount of sulphur in the

nutrient solutions for the potassium experiment. The pH of the nutrient solutions was adjusted to 6·5.

Glazed earthenware jars of uniform size (7″ × 5″) were used as the containers. These had two drainage holes at the sides towards the bottom, which were covered with acid-washed quartz particles so as to prevent the sand coming out, and the holes were closed with waxed corks from the outside. The pots were filled with the specially prepared inert sand (2,000 g.). Prior to sowing the seeds the sand cultures were flooded with the respective nutrient solutions and the excess of the solution drained off. Forty seeds were sown in each pot and on germination were thinned to 30. During the course of the experiment each pot received 2·5 litres of the nutrient solution, usually in 150 ml. lots on alternate days.

In both the nitrogen and potassium experiments there were six treatments and four replicates. The 24 pots were arranged in the glass-house as a layout of four randomized blocks in an area with uniform lighting. The experimental plants were harvested when they were four weeks old. The plants were cut 2 cm. below the cotyledonary node. Ten uniform seedlings from each pot under each treatment were chosen to determine a comparative idea of the weight of the seedlings in the different treatments. From the remaining 20 plants, a 2 g. representative sample was chosen for the vitamin C estimation. The estimations were made between 12 noon and 2 P.M.

(*a*) *Nitrogen experiment.*—This experiment consisted of six treatments with the nitrogen content varying from 0 to 67·2 p.p.m. in the nutrient solutions. The results obtained are as follows:

TABLE VII

*Showing the effect of nitrogen nutrition on the vitamin C content of* Trigonella Foenum-græcum

| Treatments N in p.p.m. | Fresh wt. in g. (10 seedlings) | Vitamin C content mg./100g. of fresh material |
|---|---|---|
| A 0 .. | 1·05 | 28·9 |
| B 8·4 .. | 1·72 | 43·4 |
| C 16·8 .. | 2·10 | 47·5 |
| D 33·6 .. | 2·22 | 55·8 |
| E 50·4 .. | 2·63 | 59·1 |
| F 67·2 .. | 2·71 | 58·9· |
| Critical difference P = 0·05 .. | 0·14 | 2·1 |
| Conclusion .. | $\overline{\text{FEDCBA}}$ or F=E> D=C> B> A | $\overline{\text{EFDCBA}}$ or E=F> D> C > B> A |

With a deficiency of nitrogen the plants showed a stunted growth and with increasing levels of nitrogen the growth was enhanced (Plate XX, Fig. 1). On fresh-weight basis a significant difference was obtained with the first five levels of nitrogen. The difference in yield with the last two highest levels of nitrogen was not significant. With respect to the vitamin C content an increase was obtained with increased levels of nitrogen in the nutrient medium upto 50·4 p.p.m., while the two highest levels gave statistically the same amount of the vitamin.

(*b*) *Potassium experiment.*—In this experiment six treatments were included the concentration of potassium in the nutrient medium varying as follows: (A) 0, (B) 5·87, (C) 11·75, (D) 23·5, (E) 35·25, and (F) 47·0 p.p.m. of potassium. The data obtained are presented in the following table.

TABLE VIII

*Showing the effect of potassium nutrition on the vitamin C content of* Trigonella Foenum-græcum

| Treatments K in p.p.m. | Fresh weight in g. (10 seedlings) | Vitamin C content mg./100 g. of fresh material |
|---|---|---|
| A 0 .. | 0·94 | 35·5 |
| B 5·87 .. | 1·35 | 38·8 |
| C 11·75 .. | 1·72 | 43·9 |
| D 23·50 .. | 2·41 | 47·9 |
| E 32·25 .. | 2·50 | 49·4 |
| F 47·00 .. | 2·53 | 48·7 |
| Critical difference P=0·05 .. | 0·08 | 2·3 |
| Conclusion .. | $\overline{FE}DCBA$ or F=E > D > C > B > A | $\overline{EF}DCBA$ or E=F=D > C > B > A |

A complete deficiency of potassium checked the growth of the plants considerably (Plate I, Figs. 2 and 3), and above 32·25 p.p.m. of potassium in the medium no significant increase could be detected on fresh-weight basis. However, below the level 32·25 p.p.m. an increase in fresh-weight was obtained with increasing potassium. Likewise a deficiency of potassium gave the lowest vitamin C content, and increase in the vitamin C content was obtained with increase in the potassium level upto 23·5 p.p.m., while higher levels of potassium did not give a significant increase in the vitamin content. With a complete deficiency of potassium in the medium a deficiency symptom was observed on the leaves of the plant; characteristic dark green colour and necrotic spots were seen on the leaves, while some of the oldest leaves had actually shrivelled up (Plate I, Fig. 3).

## Discussion

In the present studies on the vitamin C content of *Trigonella Foenum-græcum* it was observed that leaves contained about 80 per cent. of the vitamin C content of the entire plant. The leaf buds and blossom buds also showed a higher vitamin C value than the stem or roots indicating that the tissues in an active state of development contained a high concentration of the vitamin. The vitamin C content of the shoot (leafy portion) increased up to the time of flowering and gradually decreased after the flowering started. These observations are in agreement with those of Reid (1937) on the cowpea, which belongs to the same natural order as *Trigonella.*

The beneficial effect of light on the formation of vitamin C in plants has been shown by a number of investigators. In *Trigonella Foenum-græcum* it was observed that the seedlings raised outside and exposed to full sunlight contained more vitamin C than those grown in a glass-house under diffused light conditions. It was also noted that the plants which were transferred to a dark chamber for 24 hours prior to the estimation of the vitamin content contained less vitamin C than the control ones left in the open field, and that when those in the dark were removed to sunlight the plants showed an increase in the vitamin value (Table V). Similar observations have been made by a number of workers on other crops (Kohman and Porter, 1940; Reid, 1942 *a*; Virtanen and Jorma, 1945; Korajakina, 1946 *a*; Currence, 1939). Variations in the amount of vitamin C in *Trigonella Foenum-græcum* were also found at different times of the day. The vitamin content of the seedlings at midday was greater than that obtained early in the morning, and a decrease was noted by the evening (Table III). This also finds agreement with the results reported by Reid (1942 *b*), Lantz (1945), Korajakina (1946 *b*) and Hamner and Parks (1944). As emphasized by Hamner and Parks (1944), light intensities just prior to harvest appear to play a dominant role in determining the vitamin C level of vegetables, especially those of the leafy type.

Very few investigators in this country have attempted to correlate vitamin C content of vegetables with the inorganic (both macro- and micro-) nutritional amendments of soils. This work covers this field of investigation to some extent. The results obtained from the controlled sand culture experiments reported herein (Tables VII and VIII) show that, other conditions being equal, a deficiency of nitrogen or potassium decreased the vitamin C content of the leafy part of this vegetable. With an increase in these nutrient elements there was an increase in the vitamin value up to a limit (50·4 p.p.m. of N and 23·5 p.p.m. of K in the nutrient solution), after which

no significant difference in the vitamin values could be detected. The growth of the plants in the nitrogen- and potassium-deficient cultures was also similarly affected. These results are largely in agreement with recent work reported on other crops (Naik *et al.*, 1947; Petrosini, 1945; Sideris and Young, 1945, 1947). However, it should be noted that fertilizer treatments have been reported to affect vitamin C formation differently in different species of plants (Isgur and Fellers, 1937; Cross, 1939; Ferres and Brown, 1946).

The indispensability of manganese for the synthesis of vitamin C by animals and plants has been demonstrated by Rudra (1944). Rangnekar (1945) found that the formation of vitamin C in a species of *Amaranthus* was influenced to various degrees by added manganese to soil deficient in that element. The present work with *Trigonella* grown on a spent-up soil of unknown manganese content showed that an addition of a small amount of manganese (0·05 g. $MnSO_4 \cdot 4H_2O$ to 3,000 g. of soil) increased the vitamin C content of the leafy parts. However, with higher doses of $MnSO_4$ no appreciable increase in the vitamin value was obtained (Table VI). Yield on fresh and dry weight basis also showed that small amounts of $MnSO_4$ produced an accelerated growth of the plant. The beneficial effect of small amounts of manganese on the vitamin C content of *Trigonella* might perhaps be correlated with the better growth of the plant as evidenced by the increase in the fresh and dry weight of the plants. However, the importance of manganese on the vitamin C content of vegetables is a considerably debated point (Hester, 1941; Wade, 1942; Lyon and Beeson, 1943; Gum *et al.*, 1945). The present work largely indicates that traces of manganese are beneficial, and it would be interesting to study this aspect further with various vegetable crops.

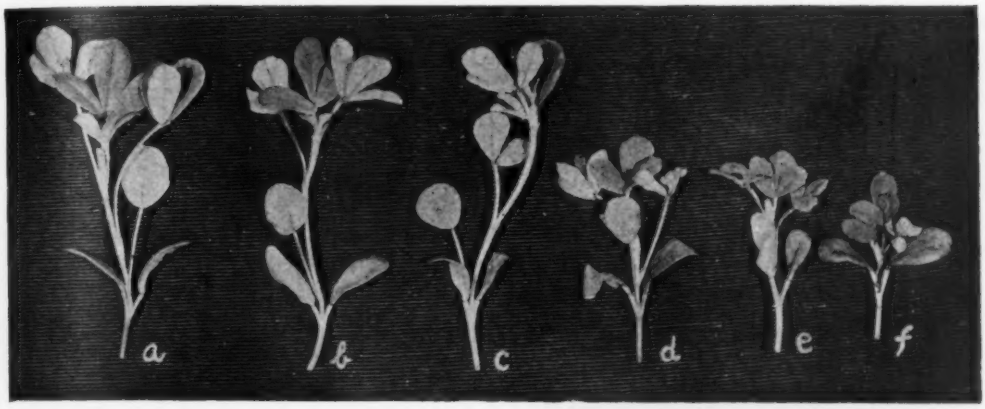
## Summary

1. A strain of *Trigonella Foenum-græcum*, a cultivated leafy vegetable, was used for the study of certain factors, *viz.*, light and mineral nutrition, governing the formation and accumulation of vitamin C (ascorbic acid) in the seedlings.

2. The localization of vitamin C in the different parts of the plant was studied. It was observed that the leaf contained a high concentration of the vitamin (about 80 per cent. of the total vitamin content), while the stem and the roots showed a very poor value. The leaf and flower buds showed a high concentration of vitamin C indicating that the tissues in an active state of development contained a high concentration of the vitamin.

3. The vitamin C content of the shoot gradually increased up to the time of flowering, thereafter a decrease in the total vitamin value was observed.

1



2

3

4. A beneficial effect of light on the vitamin concentration of the plant was noted. The plants exposed to sunshine showed a higher vitamin C value than those grown in the glass-house under diffused light conditions. Plants kept in total darkness for 24 hours contained a low vitamin C content, but on return to natural conditions in the field an increase in the vitamin value was obtained even within a short period, the vitamin concentration still being lower than that of the plants grown in the field throughout.

Variations in the vitamin C content of the seedlings were observed at different times of the day. The lowest concentration was found early in the morning, the vitamin value increasing up to 2 P.M.

5. A significant increase in fresh weight, dry weight and vitamin C content of the seedlings was obtained by the addition of small amounts of $MnSO_4 \cdot 4H_2O$ (0·05 g. per 3,000 g.) to a spent-up garden soil. Higher levels of the manganese sulphate did not show a beneficial effect.

6. A deficiency of nitrogen and potassium produced a decrease in the vitamin C content of the seedlings grown in controlled sand cultures. With increasing nitrogen or potassium an increase in the vitamin value was obtained up to a certain limit, *i.e.*, 50·4 p.p.m. of N and 23·5 p.p.m. of K in the nutrient solution. This feature also appeared to run parallel with the general growth of the plant.

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## Literature Cited

Cross, F. B. .. "The effect of certain cultural practices on the ascorbic acid content of some horticultural plants," *Thesis Univ. Missouri*, 1939 (*Biol. Abst.*, **19** ; abst. 3585).

Currence, T. M. .. "A comparison of tomato varieties for vitamin C content," *Proc. Amer. Soc. hort. Sci.*, 1939, **37**, 901–4.

Ferres, H. M., and Brown, W. D. "The effects of mineral nutrients on the concentration of ascorbic acid in legumes and two leafy vegetables," *Aust. J. exp. Biol. med. Sci.*, 1946, **24**, 111–19 (*Hort. Abst.*, **16** ; abst. 755).

Gum, O. B., Brown, H. D., and Burrell, R. C. "Some effects of Boron and Manganese on the quality of beets and tomatoes," *Plant Physiol.*, 1945, **20**, 267–75.

Hamner, K. C., and Parks, R. Q. "Effect of light intensity on ascorbic acid content of turnip greens," *J. Amer. Soc. Agron.*, 1944, **36**, 269–73.

Harris, L. J., and Olliver, M. .. "The reliability of the method for estimating vitamin C by titration against 2 : 6-dichloro-phenol-indophenol. I. Control tests with plant tissues," *Biochem. J.*, 1942, **36**, 155-82.

Hester, J. B. .. "Manganese and Vitamin C," *Science*, 1941, **93**, 401.

Isgur, B., and Fellers, C. R. .. "A preliminary study of the relationship between vitamin C content and increased growth resulting from fertilizer applications," *J. Amer. Soc. Agron.*, 1937, **29**, 890-93.

Kohman, E. F., and Porter, D. R. "Solar rays and vitamin C," *Science*, 1940, **92**, 561.

Korajakina, V. F. .. "The influence of external conditions on the accumulation of ascorbic acid in vegetables," *A symposium on scientific work carried out at Leningrad*, 1941-43, 1946 *a*, pp. 331-8. *Komarov bot. Inst. Acad. Sci. U.S.S.R. Leningrad* (*Hort. Abst.*, **17**; abst. 1771).

———— .. "The ascorbic acid content of vegetable leaves at different times of day," *A symposium on scientific work carried out at Leningrad*, 1941-43, 1946 *b*, pp. 339-44. *Komarov bot. Inst. Acad. Sci. U.S.S.R. Leningrad.* (*Hort. Abst.*, **16** ; abst. 1772).

Lantz, E. M. .. "Some factors affecting the ascorbic acid content of chile," *Bull. New Mexico agric. Exp. Sta.*, 1945, **324** (*Hort. Abst.*, **16** ; abst. 2048).

Lyon, C. B., and Beeson, K. C. "Manganese and ascorbic acid formation," *J. Amer. Soc. Agron.*, 1943, **35**, 166-67.

Naik, K. G., Shah, C. C., and Pandya, H. G. "Study on the ascorbic acid content of common fruits and vegetables of Gujarat, Part III. Effect of fertilizers on vitamin C contents of some vegetables," *Proc. 34th Ind. Sci. Cong. Absts.* Sec. **10**, abst. 12, 1947.

Petrosini, G. .. "The action of mineral fertilization on the vitamin content of plants : investigation of pepper pods I," *Ann. Chim. applicata*, 1945, **35**, 81-93 (*Chem. Absts.*, **40** ; abst. 7478).

Rangnekar, Y. B. .. "Role of manganese in the formation of vitamin C and carotene in plants," *Curr. Sci.*, 1945, **14**, 325.

Reid, M. E. .. "Localization of ascorbic acid in the cowpea plant at different periods of development," *Amer. J. Bot.*, 1937, **24**, 445-47.

———— .. "Effect of variations in light intensity, length of photoperiod and availability of nitrogen upon accumulation of ascorbic acid in cowpea plants," *Bull. Torrey bot. Club*, 1942 *a*, **69**, 204-20.

———— .. "Variations in ascorbic acid and dry matter content of cowpea plants at different times of day," *Bull. Torrey bot. Club*, 1942 *b*, **69**, 522-27.

Rudra, M. N. .. "Role of manganese in the biological synthesis of ascorbic acid," *Nature*, 1944, **153**, 743-44.

Sideris, C. P., and Young, H. Y. "Effects of potassium on chlorophyll, acidity, ascorbic acid and carbohydrates of *Ananas comosus* (L.) Merr.," *Plant Physiol.*, 1945, **20**, 649-70.

Sideris, C. P., and Young, H. Y. "Effects of nitrogen on chlorophyll, acidity, ascorbic acid and carbohydrate fractions of *Ananas comosus* (L.) Merr.," *Plant Physiol.*, 1947, **22**, 97–116.

Virtanen, A. I., and Jorma, J. .. "Changes in the ascorbic acid content of green plants in the dark," *Suomen Kemistilehti*, 1945, **18 B**, 50–2 (*Chem. Abst.*, **40**, 7310).

Wade, B. L. .. "Vitamin C content of snap bean and cabbage," *Assoc. South agric. Workers, Proc. Ann. Conv.*, 1942, **42**, 180–81. (Original not seen).

Woodman, R. M. .. "Phosphate deficiency and yield tests on sand cultures of May King lettuce," *J. agric. Sci.*, 1939, **29**, 229–49.

## Explanation to Plate XX

Fig. 1. Shows the growth of *Trigonella* seedlings with different concentrations of nitrogen in the medium.

(*a*) 67·2, (*b*) 50·4, (*c*) 33·6, (*d*) 16·8, (*e*) 8·4 and (*f*) 0 p.p.m. of N.

Fig. 2. Shows the growth of seedlings with different concentrations of potassium in the medium.

(*a*) 0, (*b*) 5·87, (*c*) 11·75 and (*d*) 23·5 p.p.m. of K.

Fig. 3. Shows a seedling grown in K-deficient sand culture (*a*) compared with one of the same age from a sand culture supplied with 23·5 p.p.m. of K (*b*). Note the necrotic spots on the leaves of the plant grown in a K-deficient medium.

# STUDIES ON THE ANÆMIA IN CHICKS INFECTED WITH *P. GALLINACEUM*

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The significance of anæmia in malaria has been emphasized by many investigators (Young, 1937; Terzian, 1941; Taliaferro and Kluver, 1940; Hewitt, 1942). In the ducks infected with *P. lophuræ* the degree of anæmia is proportional to the number of parasites in the peripheral blood. In most cases of *P. lophuræ* infection in ducks a marked drop in the total number of red blood cells occurs as the peak of parasitæmia is reached; the number of red blood cells and the level of hæmoglobin return to normal within 3–5 days following the peak of infection (Hewitt, 1942). In an untreated acute infection, death usually occurs on the 10–12 day. Hill (1942) has concluded from her studies on pigeons infected with *P. relictum* that death results from anæmia. Rigdon and Varnadoe (1945) have recently shown that life may be prolonged in ducks infected with *P. lophuræ* by repeated injection of normal duck blood.

Apparently few observations have been made on the erythrocytes in the chick and the changes they show in severe anæmia. Hewitt (1942) and Hewitt, Richardson and Seager (1942) have shown in their study of the morphology of red blood cells of malaria-infected ducks that varying degree of polychromasia occurs. The nucleii are larger than those of the mature cells and these cells may be round or elliptical. Binucleated and anucleated forms and deeply basophilic erythroblasts may also be found in the peripheral blood during severe infections. Taliaferro and Kluver (1940) have reviewed the subject and have extended our knowledge of the hæmatology of malaria in panamanian monkeys. They emphasized the occurrence of anæmia and a decrease in the amount of hæmoglobin. Normoblasts and anisocytosis were found in the peripheral blood during malarial infections in monkeys.

The physiological studies made in this laboratory owing to the similarity of the course of malaria infection in chicks infected with *P. gallinaceum* (De and Ramaswamy, 1948) have caused us to study further the changes which may occur in the erythrocytes of chicks in malaria.

## Methods and Materials

Chicks, 8–12 weeks old, were used for the purpose of this study. The strain of the malaria parasite, *P. gallinaceum*, was originally obtained from the King's Institute, Guindy, and was passed by blood inoculation through chicks of different ages and breed before being transferred to the present series under experiment. Intra-muscular inoculations have been used exclusively throughout the present work. Donor blood was drawn by cardiac puncture from two or three birds and pooled and an equal amount of citrated saline (2·0 per cent., sodium citrate, 0·9 per cent. saline) has been used as diluent.

Blood smears were stained with Giemsa and Leishmann stain. The parasitæmia was determined by counting the number of parasitized cells per 500 red cells. The erythroblast was differentiated from the erythrocytes in this study by the bluish staining of the cytoplasm and by the spherical shape of the nucleus and cell outline in comparison to the more elliptical shape of adult red cells.

The same blood smears used for determining the degree of parasitæmia were used to measure the size of different red cells. About 100 cells were measured each day during the infection to establish their average size. Measurements were made with an ocular micrometer calibrated in the usual way. A Price-Jones curve was drawn as is done in the case of elliptical erythrocytosis in man (Winston Evans, 1943). The diameters of 100 cells were measured as also the distribution of 100 cells were measured and the distribution of the cells was plotted according to the area ($\pi/4$ times the product of the long and short axis, *i.e.*, the area of an ellipse). Standard technics were used for counting the red cells. Hayem's fluid was used for the diluent. Hæmoglobin determinations were made by using Sahli's hæmoglobinometer. Smears of the spleen, liver and the bone marrow from the femur, were taken from the infected as well as normal birds and stained in the usual way.

## Experimental

The degree of parasitæmia and the severity of the accompanying anæmia varied with the age of the bird and the size of the inoculum. The course of a typical infection which ended in death is shown in Fig. 1. The degree of the anæmia is found to be proportional to the parasitæmia. Accompanying this anæmia there is a simultaneous increase in the erythroblasts in the peripheral blood. In the case of chicks the peak of parasitæmia was reached on the sixth or seventh day and then the number of parasites in peripheral blood rapidly decreased until only a few were present on the ninth day. In

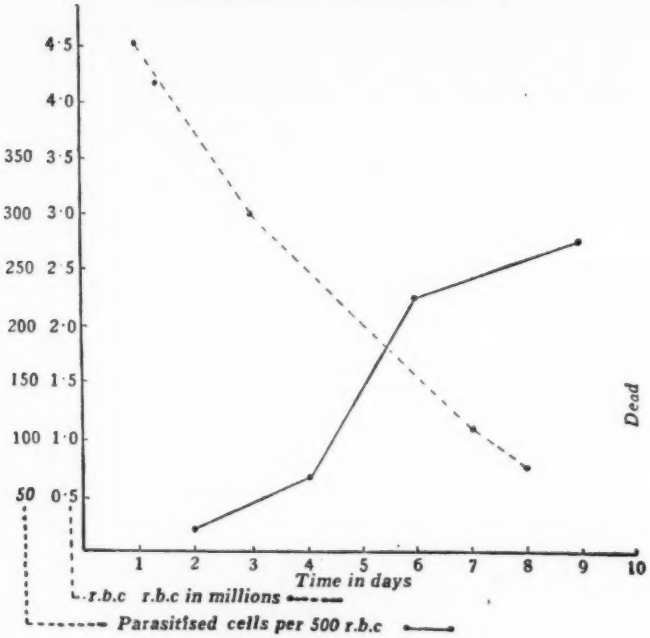


FIG. 1.

those that survived, the hæmoglobin and red cell count steadily increased from the tenth day onwards. The fall in hæmoglobin and colour index run parallel with the total number of erythrocytes in the peripheral blood (Fig. 2).

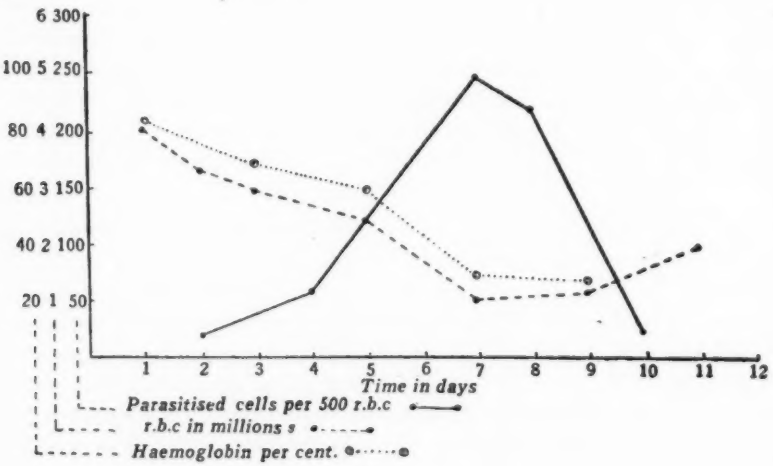
6 300
100 5 250
80 4 200
60 3 150
40 2 100
20 1 50
1 2 3 4 5 6 7 8 9 10 11 12
Time in days
Parasitised cells per 500 r.b.c
r.b.c in millions s
Haemoglobin per cent.

FIG. 2.

All the infected birds showed a marked increase in the percentage of young red cells of erythroblastic type. Near the time of death immature forms of erythrocytes enter the peripheral circulation in large numbers. Apart from this there is a variation in the size and shape of the erythroblasts found in the peripheral blood during a course of infection. These immature cells were at first elliptical in shape and slightly smaller than the adult red blood cells, but as the anæmia progressed and great strain was placed on the bone marrow, the peripheral blood was flooded with even younger cells; many of these being very small and almost spherical in shape. Typical cells seen in the peripheral blood were photographed at different intervals during the course of the infection to show the variations in their size and shape (Fig. 3).

The late erythroblasts possess a round nucleus and the particles of chromatin are larger and more compact than they are in the young type of cell. There is also a larger amount of cytoplasm which stains bluish purple. There was a marked hæmopœtic response of the bone marrow, liver and spleen, but the greatest proliferation was in the bone marrow. The bone marrow of some of the chicks showed a preponderance of early *erythroblasts*. These cells are large and round. The nucleus filling up the greater portion of the cell leaving only a thin rim of cytoplasm. Chromatin of the nucleus is fine and loosely packed. The cell measuring usually about 12–15 microns in diameter. This type of cell being very seldom seen in the peripheral blood as contrasted with the young red cell usually present in peripheral circulation. It is very interesting to note that the early erythroblasts do not have parasites with in their cytoplasm, excepting a few of the more mature erythroblasts in the peripheral blood.

The results obtained with the Price-Jones curves in chick malaria have proved somewhat anomalous. Using Leishmann stained blood films we have examined Price-Jones curves in chicks with severe malaria at a time when parasites were present in the blood. The data concerning them are given in the table below (I).

TABLE I

*Data regarding Price-jones curves in chick malaria* (P. gallinaceum)

| Case type | No. of birds | Day of infection | R.B.C. per cmm. in millions | Price-jones measurement | | |
|---|---|---|---|---|---|---|
| | | | | M.C.A. $\mu\mu$ | $\sigma\mu\mu$ | $v$ per cent. |
| Normal .. | 20 | .. | 4–4·5 | 53·44 | 2·92 | 4·43 |
| Infected .. | 20 | 5 days | 1·8–2 | 54·42 | 2·74 | 2·42 |

M.C.A. = Mean red cell area ; $\sigma$ = Standard deviation ; $v$ = Coefficient variability

## Discussion

The results of the observations made in this study are essentially comparable to those reported by Hewitt (1942) and Rigdon and Rostorfer (1947) working on *P. lophuræ* infection in ducks. The bizarre forms of red cells that may be observed in the peripheral blood may be due to pathological cells that are formed in the bone marrow finding way into the peripheral circulation. The presence of spherical erythroblasts in the blood indicates the morphological forms that a normal erythroblast of the chick undergoes before it assumes elliptical shape as a fully formed red blood cell. The bone marrow studies indicate that young red cell is round at first and the normal elliptical shape is assumed as it grows into a mature cell.

In anæmia certain physical properties of blood alter. Hamburger (1895)—Reference from Haden (1934)—observed that the erythrocytes of the horse and dog decreased in diameter as the plasma was diluted with water. Blood smears showing rouleaux formation are unsuitable for both diffraction methods (Eve's Halometer) and Price-Jones measurements but in the case of the chick these complications are not evident. So it was thought in this series, in the absence of rouleaux formation the corpuscles may not be behaving quite normally on fixation and staining. Fixation with methyl alcohol in the Leishmann stained films was for only $\frac{1}{2}$ to 1 minute, whereas in Giemsa it was in contact with methyl alcohol for 2 minutes. It may be possible that shrinkage of the corpuscle is greater on this account. It was noted in a few of the profound anæmias, where films were made simultaneously with Leishmann and Giemsa stains that the Price-Jones curves showed no marked difference. These observations are not sufficiently extensive for us to state that the anomalous results obtained are due to abnormal shrinkage of the corpuscle in stained blood films, and also a study of the red cells in the chick infected with *P. gallinaceum* is rendered difficult by the transitory nature of the hæmopœtic response. The abnormal cells are present for only a short time in the peripheral circulation and either complete recovery or death follows from the infection. Erythrocyte measurements made on normal chicks vary in size from 8·8 to 12 $\mu$ (Major axis). The nucleii of these cells were 2·8 $\mu$ to 6·4 $\mu$ (Major axis). The normal appearing erythrocytes in the peripheral blood of chicks infected with malaria are 9·6 $\mu$ to 12 $\mu$ and their nucleii 3·6 $\mu$ to 5·6 $\mu$. The more mature erythrocytes in the peripheral circulation are elliptical in shape in chicks with a low grade infection. Some of the late erythroblasts are smaller and almost spherical at the time the peak of parasitæmia is reached in the highly parasitised birds. As the degree of anæmia diminishes the late erythroblasts again become

larger and approach the shape of an elliptical body. These small round erythroblasts in chicks are found in significant numbers for 24–48 hours only among the birds that survive a severe malarial infection. It is interesting to note that the young types of erythroblasts in the peripheral blood did not have any parasites in their cytoplasm. Apparently these plasmodia do not prefer late erythroblasts to adult red cells. It is suggested therefore that one significant factor in the mechanism by which rapid decrease in the parasitæmia occurs in highly parasitized birds following the peak of infection is the result of an absence of mature erythrocytes for the parasites to enter.

Histological studies on the spleen and liver of ducks infected with *P. lophuræ* (Rigdon, 1944) have shown a proliferation of cells that were considered to be hæmopoietic tissue. The present study of the cells in the liver and spleen would indicate that these hyperplastic foci are formed primarily by cells of the red blood series. The changes that are seen in the type of red cells in peripheral blood, of course, reflect what is going on with in the blood-forming tissues. The spleen, liver and bone marrow supply young erythrocytes for the peripheral blood. Further, greatest number of red cells are formed in the bone marrow in comparison with the liver and the spleen.

## Summary

1. The study of the anæmia in chicks produced by *P. gallinaceum* shows the following characteristics. They are: (1) a slightly lowered colour index, (2) Hyperplasia of the bone marrow corresponding with a drop in the red cell count, (3) and slightly raised mean red cell area.

2. Small late erythroblasts which are spherical occur in the peripheral blood when the maximum strain is placed on the hæmopoietic tissues and the normal elliptical erythrocytes are found in the peripheral blood as the bird recovers from the acute infection.

3. It is interesting to note that the rapid reduction in the number of parasites following the peak of the parasitæmia may be related to the character of the parasites apparently preferring the erythrocytes to the erythroblasts.

## Acknowledgements

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## References


De, N. N., and Ramaswamy, A. S. .. *Curr. Sci.*, 1948, **17**, 237-38.

Haden, Russell, L. .. *Am. J. Med. Sc.*, 1934, **188**, 441-49.

Hamburger, H. J. .. *Virchows. Arch. F. Path. Anat.*, 1895, **141**, 230.

Hewitt, R. I. .. *Am. J. Hyg.*, 1942, **36**, 6-40.

——— Richardson, A. R., and Seager, L. D. *Ibid.*, 1942, **36**, 362.

Hill, Claire McDowell .. *Ibid.*, 1942, **36**, 143.

Rigdon, R. H. .. *Am. J. Trop. Med.*, 1944, **24**, 371-77.

——— and Rostorfer, H. H. .. *Blood*, 1947, **2**, 244-55.

——— and Varnadoe, Nona, B. .. *Am. J. Trop. Med.*, 1945, **25**, 409.

Taliferro, W., and Kluver, C. .. *J. Inf. Dis.*, 1940, **66**, 153-65.

Terzian, L. .. *Am. J. Hyg.*, 1941, **33** (Sect. C), 1-22, 33-53.

Winston Evans .. *J. Path. Bact.*, 1943, **55**, 378-81.

Young, M. .. *Am. J. Hyg.*, 1937, **26**, 322-36.


## Explanation of Plate

The morphological variations in the type of red blood cell that occur in the peripheral blood of a Chick that survives a severe malarial infection (*P. gallinaceum*) × 1,050. *A.* Normal erythrocytes as observed on the third day following infection. *B.* On the fifth day some of the erythrocytes are larger and others are smaller than a normal adult red cell. *C. & D.* The peak of parasitæmia occurs on the 6-7 day of infection. Small round erythroblasts are present at this period. The cells on the 7th day are frequently larger and some are elliptical in shape, while others are spherical in shape. The cytoplasm stains deep blue than it does in normal erythrocytes. *E.* The erythrocytes on the 9th day are not distinguishable from normal red cells. *F.* The normal erythrocytes appearing in large numbers on the 10-12 day of infection.

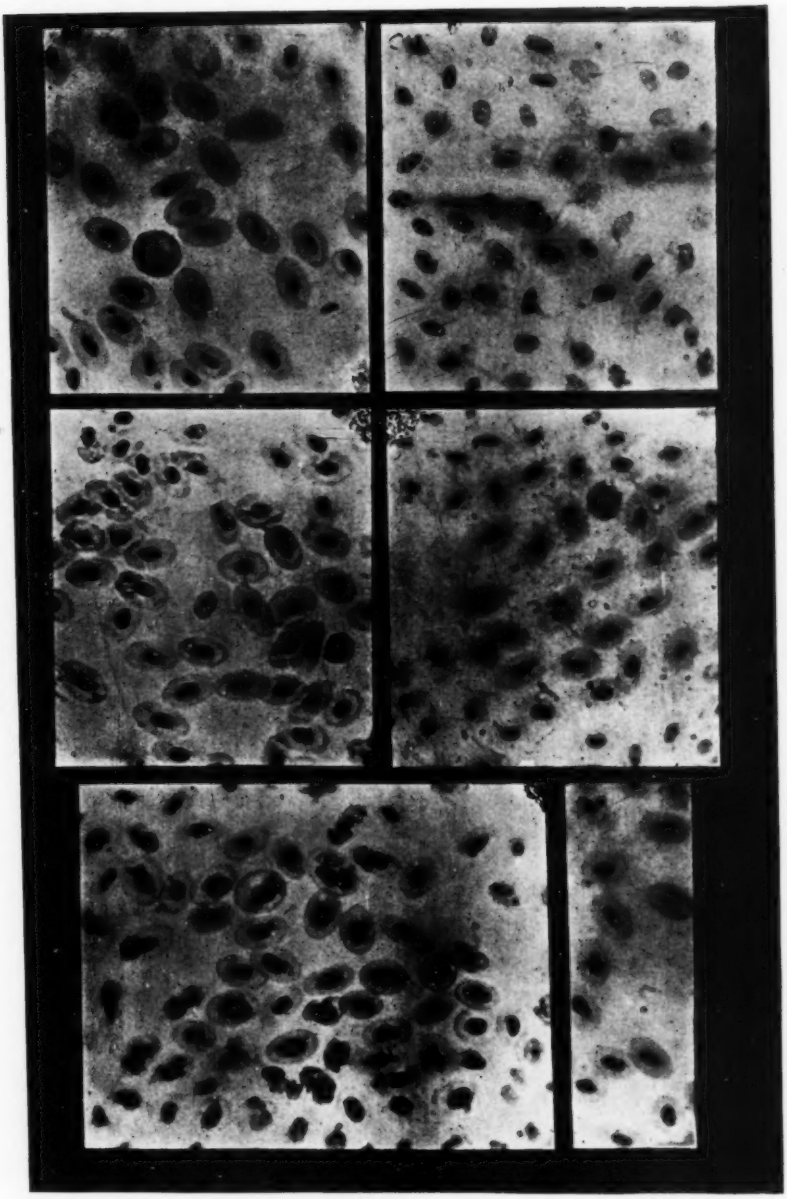


FIG. 3

# A STUDY ON THE GERMINATION OF *STRIGA* SEED AND ON THE MECHANISM AND NATURE OF PARASITISM OF *STRIGA LUTEA* (LOUR.) ON RICE

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## Introduction

In the modes of parasitism among the several phanerogamic root parasites one perhaps sees a gradual evolution from the low and primitive method of cell absorption to the more advanced and organised cell effort to secure the necessary food from the host plant. The parasite *Striga*, perhaps, comes under this latter class while *Balanophora* may be quoted as a typical example for the other. This parasite has no well-defined root or shoot system. The method of food absorption from the host is very crude and much more primitive than in the case of *Striga*. The tuberous vegetative body of the parasite corresponds to the hypocotylous segment of the seedling, the only segment that develops while all the other embryo-organs are suppressed. The ground tissue of the tuber is traversed by a woody vascular system which has now been definitely proved to be the entire roots of the host (Ekambaram and Panje, 1935) contrary to the assertions of Hooker (1855) who said that these belonged to the parasite. The host meristem penetrates into the cell-interstices of the parasite meristem which in turn pushing into the host root forms the absorptive tissue of the parasite. In *Striga* parasitic absorption of food is effected by means of conducting vessels developed inside what are known as haustoria or connecting media on the root of the parasite.

Pearson (1913) found that the germination of *Striga* seed was not possible without a host plant and that actual contact with the root of the host plant was not absolutely necessary for the seed to germinate. He is inclined to believe that the resting period necessary for the germination of the *Striga* seed varies with different seeds collected at the same time and that this might extend to anything up to 12 years. He found that an acid medium is favourable to the germination of the *Striga* seed and that the germination is greatly controlled by the physical conditions of the soil.

## Materials and Methods

1. *Germination of the Striga seed and the production of the haustorium.*—At the Agricultural Research Station at Pattambi, various experiments were conducted to germinate the *Striga* seed independently of the host plant but without any success which is in agreement with the observations of other investigators (Fuller, 1900). One-year old seeds were kept in moist dishes inside an incubator at various temperatures ranging from 30° to 40° C. but they failed to germinate. Saunders (1933) succeeded in germinating *Striga* seeds in contact with some organic excretion which he isolated from among the living roots of a maize plant which exuded it in the natural course of its metabolism. The exact nature of the influence wielded by this exudation is not fully understood at present. In the absence of a thorough investigation upon the nature of the acids present in the root hairs of rice plants, a few chemicals that ordinarily exist in the plant tissue (Ingle, 1920) were tried as contact agents to germinate the seed. The different strengths of the prepared solutions of the chemicals included 1%, 2%, 3%, 5% and 10% besides the saturation strength. 0·91% of citric acid corresponding to the acidity of the liquid contained in the root hairs of a large number of plants as recommended by Dyer (1894) was also used. The substances tried were carbohydrates like glucose, fructose, saccharose and gum-arabic. None of these induced any germination of the seed. A medium containing 4% agar with 10–40% saccharose also gave negative results. Among the organic acids tried were formic, acetic, stearic, oxalic, tartaric, malic, succinic and tannic acids. These, as well as glycerol, had no effect on the seed. The inorganic acids tried included sulphuric, nitric and hydrochloric acids. None of these helped the seeds to germinate. It was at first considered that the failure of germination was due either to the fact that the physical conditions arising in the soil were not obtainable in the laboratory or that the seed had not passed the necessary dormant or resting period. The second assumption had, however, to be ruled out, since seeds varying in age from 9 months to 5 years readily germinated when sown with rice seed in pot culture. Pearson (1913) managed to germinate *Striga* seed in association with maize within 2 months after it was collected. There is a certain amount of indirect evidence to indicate that the former assumption is also not correct. For instance, even in infected fields there is no appearance of *Striga* in the interspaces and alley ways of experimental plots which do not have any rice plant growing there. Thus the only logical inference was that the germination of the *Striga* seed is possible only under a stimulation imparted to the seed when the latter came into contact with the root of the host plant.

In an earlier investigation by the writer, it had been concluded from observations on the comparative sizes and development of the primary haustorium, plumule and radicle, that the formation of the haustorium might probably be considerably in advance of the radicle and plumule. This was later confirmed by pot-culture experiments, using specially devised boxes to germinate the *Striga* seed. The device adopted consisted of a tapering box with glass panes on three sides very similar to a trader's case. In a later modification the glass plate was horizontally cut into several smaller pieces which could still be aligned into a single plate by putting one piece over the other, each piece being fastened at the ends to the sides of the box. This arrangement facilitated the removal of the panes at any particular height for observation inside the soil directly opposite to it. The box was filled with soil and paddy sown on 2–4–1936. On 29–7–1936 a few *Striga* seeds were dusted on the root tip of a lateral root of rice adhering to the inside surface of one of the glass pieces by gently removing it. After dusting the seeds the glass piece was replaced in its original position. The seeds were kept under daily observation. On 5–8–1936 a few seeds were found to have produced the primary haustoria. It was further noticed that none of the seeds deposited away from the absorptive region of the root produced any sucking organs. It is reasonable, therefore, to suppose that the absorptive region in the root alone exudes the stimulant necessary for the germination of the *Striga* seed. This clearly establishes that the haustorium in the *Striga* seed grows much in advance of the plumule and the radicle, leading one to infer that the provision in the minute seed of *Striga* of stored food is intended mainly for the germination and sustenance of the haustorium the host plant being absolutely indispensable for the parasite even from its very early stage.

2. *Haustorium and the nature of parasitism.*—It is well known that *Striga* accomplishes the function of parasitism by establishing root connections with the host plant by means of what are known as haustoria or sucking organs developing in the roots of the parasite. The haustorium which establishes this vital connection between the host and the parasite for the first time is known as the primary haustorium. As the parasite grows, secondary haustoria develop on its roots seeking further connections with the host to meet the growing demands of the parasite.

In almost all cases of haustorial attachment to the rice root examined, all of the youngest haustroria without exception were found confined to the tender root ends of rice. This is amply borne out by the experimental evidence deduced elsewhere in this paper to show that *Striga* seeds in contact

with rice root produced haustoria only when these happen to come within the absorptive region of the root. Thus the same exudation which is supposed to impart the necessary stimulus to activate the primary haustorium, is probably responsible for the birth and growth of the secondary haustorium. What appear to be normal and free roots of a full grown *Striga* plant are evidently not strictly intended for the normal physiological functions of a regular root. These are apparently, only a provision for helping the plant to secure as many connections as possible with the roots of the host. The simple experiment recorded elsewhere (Uttaman, 1949) proving the helplessness of *Striga* when divorced of its host, lends support to this view. Again, during a study of the histology of the haustorium referred to later in this paper, sufficient evidence has been collected to prove that *Striga* does not cease its vital connections with the host plant even after emergence from the soil. It is not uncommon to find as many as six or more haustorial connections in the same root on account of the several contacts with the rice root when they happen to run parallel to each other. Luthra (1921) in the course of his studies of *Striga densiflora* on sugarcane, thinks that this parasite absorbs the food materials from the soil as a normal course to elaborate its own food while being parasitic on the host. *Striga lutea* parasitic on rice does not seem to behave in a similar manner. It possesses all the essential features of a real parasite while under the soil and the look of a truly self-supporting plant above the soil with its own green leaves and the normal looking system is only apparent. Therefore to the extent to which it prefers to be free of the host plant for the preparation of its food out of the raw materials supplied by the host, it may be called partial in its nature of parasitism.

3. *The histology of the haustorium.*—It is now an accepted fact that the haustorium is brought into being as a result of some stimulus imparted to the root of the parasite by some natural exudation flowing from the host root and coming into contact with the former. A study of a transverse section of a *Striga* root through a haustrorium near its periphery (Figs. 1 and 2) discloses several interesting facts. It is apparent that the young haustroium takes its origin from the cortex adjoining the pericycle which is a single layer of cells. The larger cells nearer the pericycle and the smaller ones in the outer layers (Fig. 2) indicate that these cells lying close to the pericycle are meristematic in action and are derived from the pericycle. The haustorium takes its origin from these cells which by intense multiplication push the overlying collenchymatous cells made up of about 4 to 5 layers bordered by the outermost piliferous layer. The spongy parenchymatous tissue composed of very minute protoplasmic cells located in the cortical

region between the exodermis and the pericycle is indeed the ground meristem, a few of whose specialised cells by further activity multiply by tangential division, piling one upon the other in linear fashion (Fig. 3). These superposed cells by dissolution of their partition walls at a later stage constitute the several sucking vessels or hyphæ inside the haustorium (Fig. 5). The alignment of the cells prior to their transformation into vessels is so directed as to connect them with the main conducting vessels in the mother root.

4. *The conducting vessels in the haustorium compared to the rootlets in a normal plant.*—The functional organisation initiated by the procambial cells resulting in the creation of the several conducting vessels within a haustorium does not aim at production of a more complicated system than that of several simple vessels mostly of pitted and reticulate nature (Fig. 5). There is no secondary meristem originating from the procambium in evidence and therefore there is no further development of these simple vessels into a more complicated system of vessels. In a transverse section of the haustorium fused with the host root (Fig. 5), the main sucking vessles or the invading hyphæ within the haustorium, numbering in all appearance six, reach well nigh half the way inside the haustorium. Thereafter they branch out into a large number of plain hyphæ which intertwine and coalesce into one another and finally emerge to fuse with the conducting vessels of the host plant. The whole phenomenon offers a close analogy to the root system of a normal flowering plant. The many plain looking pitted vessels that have branched out from the main vessels and fused with the vascular bundles in the host plant can thus be compared with the several root hairs in the absorbing region of an ordinary root. The main vessels too are pitted and reticulate and look in all essentials simple xylem vessels. To confirm if these are branches in continuation of the xylem vessles (Fig. 4) of the *Striga* root and incidentally to ascertain their number, transverse sections were made of a pretty old lateral root of *Striga* through a plane in the close neighbourhood of a haustorium. The section (Fig. 6) when double-stained with Safranin and Light Green indicated the xylem vessels to be six in number.

5. *The mechanism of food absorption from the host plant.*—C. J. Pierce (1893) by his studies on the formation and development of the haustorium in *Cuscuta americana*, a parasite on *Thevetia nerrifolia*, has put forward certain hypothesis. This probably applies to *Striga* as well with some reservations. Figure 5 representing a transverse section of a haustorial attachment in the course of a parallel alignment of both the *Striga* and the rice roots represents, more or less, the mechanism of the parasitism of *Striga* on rice. The section shows very clearly the development of the conducting vessels,

both the pitted and the reticulate, in the haustorium surrounded by thin parenchymatous cells. These vessels evidently are the tributaries to the main conducting vessels in the root of *Striga*, the free ends effectively fusing with the vascular bundles in the rice root. By the growth of the haustorial hyphæ, the several layers of the parenchymatous cells and the epidermal layers overlying these are elevated into a curved cushiony structure (Fig. 5) which acts as a pre-hensile organ. It is presumed that this appendage or haustorium, by the combined effect of the high pressure brought about by the rapid multiplication of the procambial cells giving rise to it and the solvent action of some exudation through the thin walls of the parenchymatous cells overlying it, effects its passage first through the epidermal wall and later through the cortical region of the host root. Meanwhile, the growth and development of the invading hyphæ within the haustorium, by the combined effect of the rapid division and multiplication of the cells, keep pace with the external growth of the haustorium. They first effect their passage through the few overlying layers of the parenchymatous cells and then through the epidermal cells of the haustorium. By the time the latter had reached the endodermic cells whithin the host tissue, the haustorial hyphæ force their way into the stelar region of the host, breaking through the last of their barricades, namely, the endodermic layer and the rest (Figs. 7 and 8). Here the parasite enters the second stage of offensive in its drive against the host. From here onwards the haustorial hyphæ take up a more prominent stand in the parasitic raid of *Striga* against its host. The outermost cell or the hyphal tip is so adapted in form and shape as to effectively surround or probe into the particular host vessel which it chooses to pillage (Fig. 9).

Stephens (1912) and Saunders (1933) were unable to find any sieve tubes in the haustoria of *Striga*. This is confirmed by the writer's own investigations. In the absence of anything like a sieve tube among the haustorial vessels, which in all respects resemble simple roots, it is reasonable to suppose that these could connect with any kind of vessels in the host, whether phloem or xylem. Figures 10 and 11 adduce sufficient evidence to prove that this is actually the case. Further, as would be expected, it is seen that the choice between the phloem and the xylem vessels is, in fact, mainly determined by circumstances. The transverse section of a primary haustorium in fusion with the root of rice while the young *Striga* was still underground and therefore absolutely dependent on the host plant, discloses parasitc connections with the phloem vessels (Fig. 10), whereas, in the section made of a secondary haustorial connection in the root end of a tender lateral root of rice, evidently effected after the parasite had emerg-

ed from the ground, it is seen that the connection is with the xylem vessels (Fig. 11). This probably adds to the evidence advanced in this paper to prove that the *Striga* in its subterranean life lives the life of a total parasite and even later after it emerges from the soil, does not cease to be a parasite. The only difference is that in the later stages, the parasitism becomes slightly modified in character, in that it prefers to seek only the crude materials from the host and not the elaborated food. The conducting vessels in the haustorium serves the function, therefore, of both the phloem and the xylem vessels at some time or other during the life of the parasite.

Mary G. Thoday (1911) in her studies on *Cuscuta* has demonstrated that in the fusion with the sieve tubes, the invading hypha takes care to lay itself against a sieve area alongside a sieve tube, in order to dissolve by hydrolysis the hyphal cell wall at the point of contact, preferring not to injure the mechanism and thereby impair the functional efficiency of the host generally. It appears from Fig. 11 that the haustorial hypha bores straight into the xylem vessel injuring its structure considerably in its attempt to establish the parasitic connection with the host. The phenomenon observed in the case of *Striga* on rice seems to be, therefore, not in line with what has been demonstrated to be the case in *Cuscuta*. The mechanical injury done to the host vessel by the haustorial hypha in its parasitic raid appears, therefore, partly to contribute to the injury sustained by the host plant on account of the parasite.

## Summary and Conclusion

1. It has not been possible to germinate *Striga* seed independently of the host.

2. In a *Striga* seed lying in contact with the rice root, the primary haustorium grows in advance of the plumule and the radicle.

3. Only the absorptive region of the host root exudes the stimulating substance necessary for the germination of the *Striga* seed.

4. The same exudation which is supposed to impart the necessary stimulus to activate the primary haustorium is probably responsible for the birth and development of the secondary haustorium.

5. The roots of *Striga* do not seem to perform the normal physiological functions of the regular root. Apparently they serve only to help the plant in securing as many connections as possible with the roots of the host plant.

6. As in *Cuscuta* the haustorium arises from the meristematic cells of the inner cortex.

7. The haustorial branching vessels are comparable to the rootlets of a normal plant.

8. The main conducting vessels, six in number in the haustorium, appear to be continuations of the main xylem vessels in the root of *Striga*.

9. The haustorium enters the host as far as the endodermis only. Further progress leading to the attack in the stelar region is made by the invading vessels inside the haustorium.

10. There are no sieve tubes traceable in the haustorium.

11. For the haustorial vessels to get parasitic connections either with the phloem or with the xylem vessels appears to be mainly conditioned by circumstances. In the case of an underground *Striga* the connection is with the phloem while this is with the xylem when the plant has emerged from the ground. This adds to the evidence already advanced that *Striga* behaves as a total parasite when it is under the ground while above ground it is partial in its parasitic nature.

12. The conducting vessels in the haustorium serve the functions of both the phloem and the xylem.

13. The mechanical injury done to the host vessels by the haustorial hypha in its parasitic raid also appears to contribute to the injury done to the host plant by the parasitism of *Striga*. This phenomenon does not seem to be in line with what has been observed in the parasite *Cuscuta* in which the invading hypha fuses with the host vessel in such a way as not to cause it any injury.

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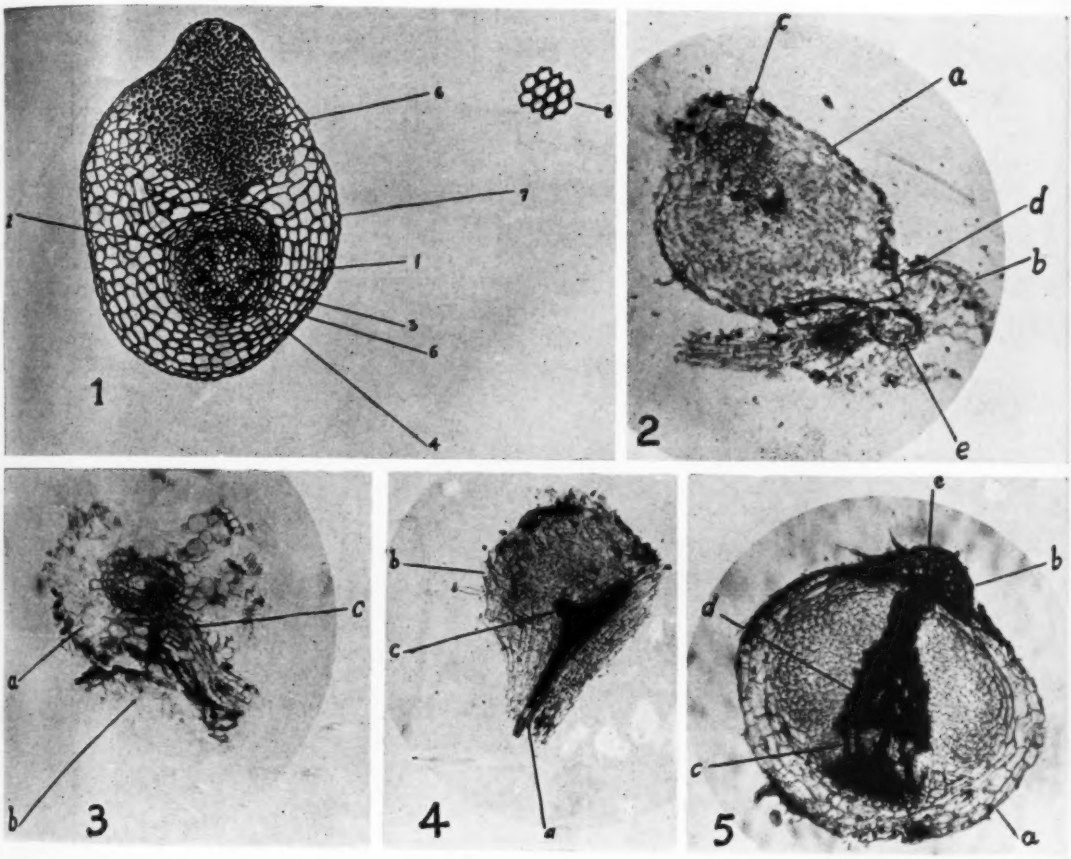
## References

1. Dyer .. "On the analytical determination of probably available mineral plant food in soils," *Trans. Jour. Chem. Soc.*, 1894, **65**, 115.

2. Ekambaram, T. S., and Panje "Contributions to our knowledge of *Balanophora*—The morphology and parasitism of the tuber," *Proc. Ind. Acad. Sci.*, 1934-35, **B 1**, 452.

3. Fuller, C. .. "Rooiblœm or witch weed," *Agri. Jour. Union of S. Africa*, 1911, **1**, 684-85.

4. Hooker, J. D. .. "The Structure and Affinities of Balanophoraceæ," *Trans. Linn. Soc.*, 1855, **22**, 1.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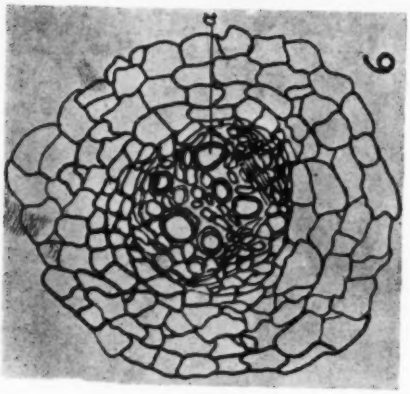
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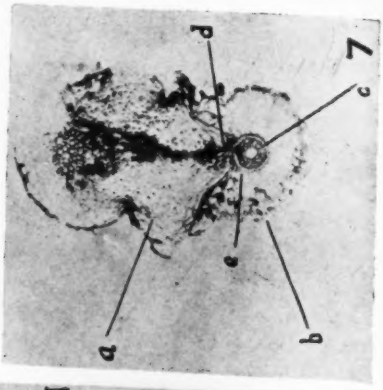
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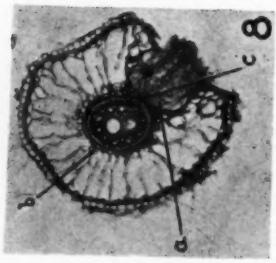
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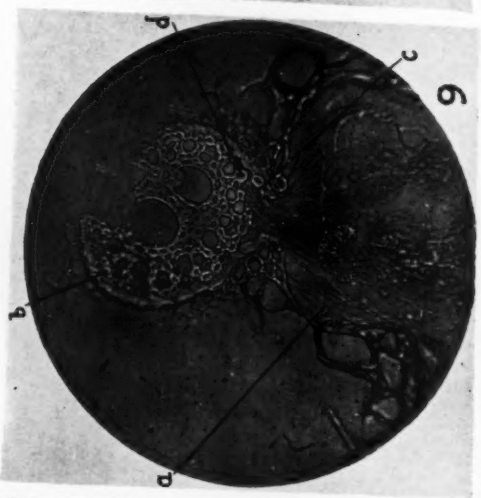
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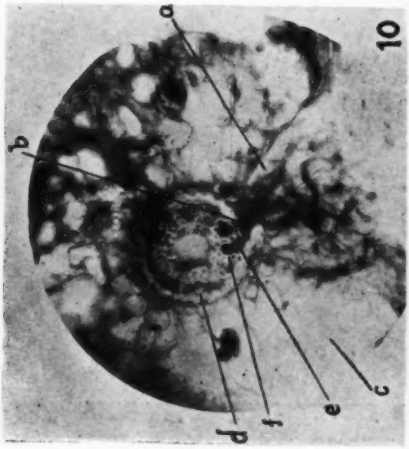
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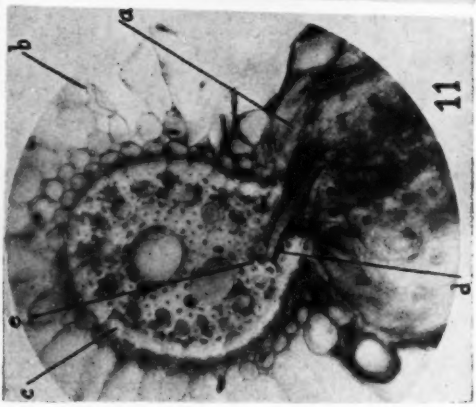
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b
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5. Ingle, H. .. *Manual of Agri. Chemistry*, 1920, 213.

6. Pearson, H. H. W. .. "On the Rooiblœm," *Agri. Jour. Union of S. Africa*, 1912, **3**, 651–55.

7. ———— .. "The problem of the witch weed," *Bull. Dep. Agri., S. Africa*, 1913, **40**, 3–34.

8. Pierce, G. J. .. "On the structure of the haustoria of some phanerogamic parasites," *Ann. Bot.*, 1893, **7**, 291–327.

9. Saunders, A. R. .. "Witch weed," *Farming in S. Africa*, **5**, 267, S'30.

10. Stephens, E. L. .. "The structure and development of haustorium of *Striga lutea*," *Ann. Bot.*, 1912, **26**, 1067–76.

11. Thoday, G. .. "On the histological relations between *Cuscuta* and its host," *ibid.*, 1911, **25**, 655–82.

12. Uttaman, P. .. "Parasitism of *Striga lutea* (L.) on rice and methods to protect rice plant against *Striga*," *Mad. Agri. Jour.*, 1950, **37**, 99–118.

## Description of Plates

Fig. 1. Transverse section of root of *Striga* passing through a fairly old haustorium a little away from the haustorial hyphæ within, to show the inner structure and origin of the haustorium as seen through the microscope under low power. 1. Xylem; 2. Phloem; 3. Cambium; 4. Pericycle; 5. Endodermis; 6. Ground tissue of very minute, thin-walled protoplasmic cells; 8. Cells in (6) magnified.

Fig. 2. Microphotograph of the transverse section of a haustorium in the early stages of attack on a rice root. Note it has reached only half the way inside the cortex of the host root. *a*. Section of the haustorium (Note the larger cells nearer the stele of the *Striga* root); *b*. Section of the rice root; *c*. Stele of the *Striga* root; *d*. Superior end of the haustorium; *e*. stele of the rice root × 70.

Fig. 3. Microphotograph of the transverse section of a haustorium in fusion with the rice root showing the haustorial vessels in the making. *a*. Section of the rice root; *b*. Section of the superior end of the haustorium; *c*. The row of cells with their septal walls intact prior to their dissolution before transforming into haustorial vessels.

Fig. 4. Microphotograph of the longitudinal section of a *Striga* root bearing a haustorium to show the haustorial vessels branching out from the conducting vessels in the mother root. *a*. Conducting vessels in the root of *Striga*; *b*. Haustorium; *c*. Haustorial vessels.

Fig. 5. Microphotograph of the transverse section of a haustorium in fusion with the root of rice showing the mechanism of food absorption by the parasite from the host root. *a*. Section of the haustorium; *b*. Section of the rice root; *c*. Main haustorial vessels as having branched out from the conducting vessels of the *Striga* root; *d*. Sub-branches from the main vessels of the haustorium; *e*. Fusion of the haustorial vessels with the conducting vessels of the host root.

Fig. 6. A camera-lucida sketch showing the transverse section of a fairly old root of *Striga* in the close neighbourhood of an haustorial attachment. *a*. Xylem × 235.

Fig. 7. Microphotograph of the transverse section of an entire haustorium in fusion with the rice root to show that the haustorium attacks and clears its way in the host tissue as far as the endodermis only. *a*. Haustorium; *b*. Section of the rice root; *c*. Stele of the rice root; *d*. Hyphal tube in the haustorium penetrating the stele; *e*. Endodermis of the rice root × 60.

FIG. 8. Microphotograph of the transverse section of a haustorium in fusion with the rice root magnified to show that the haustorium clears its way into the host tissue as far as the endodermis only. *a*. Tip end of the penetrating haustorium ; *b*. Endodermis in the rice root ; *c*. The invading hypha in the haustorium penetrating the endodermis × 70.

FIG. 9. A highly magnified microphotograph of the transverse section of a haustorium in fusion with the rice root showing the mode of attack of the host vessel by the haustorial hypha. *a*. Tip end of the penetrating haustorium ; *b*. Stele of the rice root ; *c*. Haustorial hypha with the tip adapted to surround a xylem vessel (*d*) in the host tissue × 210.

FIG. 10. A highly magnified photomicrograph of the transverse section of a haustorium that had fused with the rice root before the *Striga* emerged from the ground. The photograph shows the fusion of the haustorial hyphæ with the phloem vessels in the host tissue. *a*. Tip end of the penetrating haustorium ; *b*. Haustorial hypha ; *c*. Cortex of the rice root ; *d*. Stele of the rice root ; *e*. Phlœm ; *f*. Xylem × 200.

FIG. 11. A highly magnified photomicrograph of the transverse section of a haustorium that had fused with the rice root after the *Striga* had emerged from the ground. The photograph shows the fusion of the haustorial hypha with the xylem vessel of the host root. *a*. Tip end of the penetrating haustorium ; *b*. Cortex of the rice root ; *c*. Stele of the rice root ; *d*. Haustorial hypha whose pre-hensile tip has bored into the xylem (*e*) of the host × 265.

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# BIOSYNTHESIS OF VITAMIN C DURING GERMINATION

## I. Effect of various environmental and cultural factors

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VARIOUS treatments, physical, chemical and biological, have been reported to affect biogenesis of vitamin C in germinating seeds as well as in growing plants. Of especial interest have been the observations that $Mn^{++}$ stimulates ascorbic acid formation and that sugars, especially glucose and mannose, serve as possible precursors. There is however considerable disagreement in the observations of different workers. In view of the very definite and recent advances in the chemical methods of determining ascorbic acid, making these methods specific for the vitamin and excluding artefacts and other reductones, the following re-investigation of the changes in vitamin C and related factors, as brought about by germination of legume seeds over a variety of conditions, was undertaken.

### EXPERIMENTAL

*Materials and Methods.*—The legumes used in these studies were *müng* (*Phaseolus radiatus*), Bengal gram (*Cicer arietinum*), Peas (*Pisum arvense*), *Masöör* (*Lens esculenta*) and *Matki* (*Phaseolus aconitifolius*). Seeds, after overnight soaking, were germinated inside Petri dishes lined with absorbent cotton wads kept moistened by periodical watering. Unless otherwise stated, these dishes were housed inside glass-walled humid chambers which were well ventilated and had an environmental temperature of $27° \pm 2°$ C. All germinated seedlings sampled for analysis were free from any apparent microbial or other contamination. The period of germination has been counted starting from the day following this overnight period of soaking.

Representative samples of germinated seedlings at various stages of growth were taken for analysis; moisture determinations were made on separate lots sampled simultaneously from the same dish. Ascorbic acid was determined by micro-titration using Tillman's dye according to the method of Harris and Olliver.[1] Frequently, the values were checked by the procedure of Mapson[2] using formaldehyde. Dehydro ascorbic acid

and total ascorbic acid were determined by Mapson's method.[2] The results are given in mgms. per 100 gms. on dry basis and represent the averages of duplicate determinations from each of at least two independent lots. No definite conclusions have been made except where replicates have borne them out.

Maximum elaboration of ascorbic acid in *müng* seeds was generally observable on the 5th day of germination. Thus, the vitamin contents of seedlings (mgms. per cent.) were 40·4, 71·0, 97·2, 120·2, 134·5 and 125·0 respectively during the first six days; at about this period, the seedlings withered and there was a drop in ascorbic acid values. Occasionally, depending on the season, the period of maximum ascorbic acid formation varied within a day, the extent of such difference being 5–10 per cent. from the values for the preceding and following days.

### *Effect of* $Mn^{\cdot\cdot}$ *Treatment*

Seeds were soaked overnight in solutions of manganese sulphate of $Mn^{\cdot\cdot}$ concentrations 10, 50, 100 and 150 p.p.m. They were then germinated as usual, water being added periodically as needed. A second series of experiments differed from the above in that waterings were made with solutions of manganese sulphate of respective $Mn^{\cdot\cdot}$ strengths. This latter method, which has been followed by other workers, would imply increased effective concentration of $Mn^{\cdot\cdot}$ with each addition of the salt solution.

A typical set of results with *müng* seedlings sampled for analysis on the fifth day is given in Table I.

TABLE I

*Effect of* $Mn^{\cdot\cdot}$ *treatment on germinating* Müng

| | Concentration of manganese ion | | | | |
|---|---|---|---|---|---|
| | Nil | 10 p.p.m. | 50 p.p.m. | 100 p p.m. | 150 p.p m. |
| | Ascorbic acid in mgms. per 100 gms. | | | | |
| 1st series .. | (i) 128·4 | 133·1 | 140·0 | 132·9 | 119·0 |
| | (ii) 128·9 | .. | 129·4 | 121·9 | 109·6 |
| 2nd series .. | (i) 123·4 | 130·2 | 127·5 | 129·4 | .. |
| | (ii) 130·7 | 131·5 | 137·2 | 129·8 | 134·0 |

Manganese treatment by the second procedure was probably excessive and hence any small increased formation of ascorbic acid as a result of manganese addition, especially in low concentrations, is not observable in this series as much as in the first series where manganese salt was not used for subsequent waterings. Similar results were obtained with other legumes. In the following studies on the effect of manganese treatment, only the technique described in the first series was adopted.

TABLE II

*Effect of $Mn^{++}$ treatment on changes in ascorbic acid in germinating* Müng

| Day of germination | Concentration of manganese ion | | | | |
|---|---|---|---|---|---|
| | Nil | 10 p.p.m. | 50 p.p.m. | 100 p.p.m. | 150 p.p.m. |
| | Ascorbic acid in mgms. per 100 gms. | | | | |
| 1 | 41·6 | 40·5 | 37·5 | 35·2 | 40·0 |
| 2 | 77·0 | 79·4 | 82·6 | 78·4 | 83·2 |
| 3 | 99·5 | 104·9 | 108·0 | 105·8 | 103·4 |
| 4 | 109·9 | 113·7 | 115·0 | 113·8 | 113·3 |
| 5 | 120·5 | 119·8 | 120·8 | 119·9 | 115·4 |
| 6 | 106·7 | 111·0 | 112·6 | 101·8 | 101·7 |

In Table II are given the results obtained when seedlings were sampled every 24 hours. This was felt desirable because treatment with $Mn^{++}$ might have altered the metabolic phase of the plant with possible maximum formation of the vitamin on different days. However $Mn^{++}$ treatment does not seem to have stimulated ascorbic acid synthesis. The average per cent. increases in the vitamin as a result of $Mn^{++}$ additions in concentrations of 10, 50, 100 and 150 p.p.m. over five-day period of germination were, respectively, 2·12, 3·34, 1·03 and 1·56.

With 50 p.p.m. of $Mn^{++}$, the per cent. differences in ascorbic acid, dehydro ascorbic acid and total ascorbic acid over untreated *müng* seedlings are as below (Table III).

The average increases in ascorbic acid, dehydro and total ascorbic acid over the 7-day period as a result of treatment with $Mn^{++}$ were $+1·39$, $-5·13$ and $+0·46$ respectively.

TABLE III

*Changes in ascorbic acid, dehydro ascorbic acid and total ascorbic acid* (50 p.p.m. $Mn^{++}$)

| Day of generation | Percent difference over untreated seedlings | | |
|---|---|---|---|
| | Ascorbic acid | Dehydro ascorbic acid | Total ascorbic acid |
| 1 .. | −6·37 | Nil | −6·37 |
| 2 .. | −4·32 | + 8·00 | −4·21 |
| 3 .. | +6·64 | + 3·58 | +6·39 |
| 4 .. | +5·56 | −11·81 | +2·24 |
| 5 .. | −0·53 | −16·25 | −0·61 |
| 6 .. | +1·06 | + 1·81 | +1·20 |
| 7 .. | +2·69 | − 9·75 | Nil |
| 8 .. | +5·53 | −14·49 | +0·54 |

The distribution of the vitamin in the foliage (true or primary) leaves, cotyledons and the remaining portion of the seedlings (the stem or the stalk), sampled on the 5th day of germination is shown in Table IV.

TABLE IV

*Distribution of ascorbic acid in the seedling (mgs. %)*

| | Untreated | | Treated | | Percent difference in Total ascorbic acid |
|---|---|---|---|---|---|
| | Ascorbic acid | Dehydro-ascorbic acid | Ascorbic acid | Dehydro-ascorbic acid | |
| Leaves .. | 491·5 | 14·3 | 500·9 | 14·1 | +1·82 |
| Cotyledons .. | 86·3 | 25·8 | 82·3 | 24·0 | −5·17 |
| Stem .. | 125·8 | 21·5 | 127·5 | 19·8 | Nil |

While biosynthesis is maximum in the leaves, $Mn^{++}$ treatment failed to enhance significantly ascorbic acid or dehydroascorbic acid formation either in the whole seedling or in its parts.

*Effect of seed treatment with magnesium and borate ions*

The results obtained with overnight soaking in solutions of magnesium sulphate (0·05 per cent. ≡ 10 p.p.m. $Mg^{\cdot\cdot}$) and boric acid (0·01 per cent.) are shown in Table V.

TABLE V

*Effect of* $Mg^{\cdot\cdot}$ *and* $BO_3'''$ *treatment* (Müng *seedlings*)

| Day of germination | Percent difference in ascorbic acid over untreated | |
|---|---|---|
| | Magnesium ion | Borate ion |
| 3 .. | +0·29 | +3·34 |
| 5 .. | +0·72 | +9·64 |
| 7 .. | −0·94 | +5·78 |

While treatment of seeds with $Mg^{\cdot\cdot}$ in the concentration used by Shen *et al.*[3] is ineffective in bringing about any change in the synthesis of ascorbic acid by germinating seeds, treatment with boric acid in the concentration employed here had a slight stimulating effect on ascorbic acid formation by seedlings (*cf.* Ahmed *et al.*[4]).

*Effects of fertilizer salts*

Salts of potassium, nitrogen and phosphorus were studied for their influence on the biosynthesis of ascorbic acid by seedlings in view of their importance as fertilizers. The combined effect of nitrogen, potassium and phosphorus

TABLE VI

*Effect of salts on ascorbic acid synthesis in* Müng *seedlings*

| Treatment | | Percent difference in ascorbic acid over untreated | |
|---|---|---|---|
| | | 3rd day | 5th day |
| Ammonium sulphate | (0·02M) .. | −33·41 | −21·28 |
| Potassium nitrate | (0·02M) .. | −25·34 | −15·52 |
| Urea | (0·02M) .. | −12·19 | − 4·74 |
| Disodium hydrogen phosphate | (0·02M) .. | −15·45 | − 9·43 |
| Disodium hydrogen phosphate + Potassium nitrate | (0·04M of each) .. | −42·26 | −23·72 |

was also studied. *Müng* seeds were soaked overnight in the salt solutions as indicated in Table VI (*cf.* Mapson *et al.*[5]) and then germinated as usual.

Most of the treatments were somewhat drastic in character though varying in degree. All the inorganic additions resulted in lowered ascorbic acid production as compared to untreated seedlings on 3rd day, this effect tending to disminish later.

*Effect of treatment by steeping in the cold*

It was observed that, when seeds were soaked overnight prior to germination at different above-room temperatures, there were differences in the extent of ascorbic acid formation. Thus, with soaking at 37° C., there was complete inhibition of germination; at 34° C. there was 15 per cent. higher ascorbic acid formation in the five-day seedlings when compared to those steeped at room temperature (26°–28° C.). At 32° C. the difference was only slight.

In the following experiments, the effects of cold treatment were studied. *Müng* seeds were given a preliminary soaking in water for different periods inside a refrigerator ($0 \pm 1°$ C.); they were then germinated as usual on cotton wads inside Petri dishes. Control seeds were germinated without this cold treatment during soaking. Representative samples were taken for analysis of ascorbic acid (Table VII).

TABLE VII

*Effect of steeping* Müng *seeds at* 0° *C. prior to germination*

| Seeds steeped for (hours) | Germinated for (days) | Ascorbic acid in | | |
|---|---|---|---|---|
| | | Untreated seeds mgs. % | Treated seeds mgs.% | % difference |
| 24 | 4 | 110·9 | 123·8 | +11·63 |
| 48 | 4 | 110·9 | 91·2 | −17·77 |
| 6 | 5 | 143·0 | 158·1 | +10·76 |
| 24 | 5 | 143·0 | 176·3 | +23·29 |
| 30 | 5 | 149·8 | 193·8 | +29·37 |
| 48 | 5 | 149·8 | 151·4 | + 1·06 |
| 24 | 6 | 127·3 | 148·7 | +16·81 |
| 48 | 6 | 127·3 | 134·1 | + 5·34 |

Pre-treatment during soaking prior to germination at low temperature had a definite favourable effect on ascorbic acid formation, a maximum difference of about 30 per cent. being observable after 30 hours steeping in the cold; prolonged periods of steeping in the cold were not beneficial. Soaking prior to germination is normally done overnight, but the differences reported here are attributable only to the low temperature and not to the combined effects of temperature and darkness (during the period of steeping), the latter factor existing even in untreated seeds. These data have been verified by independent determinations of ascorbic acid by Mapson's procedure. As with seedlings germinated in diffused light, $Mn^{++}$ (50 p.p.m.) had no effect on the formation of ascorbic acid in seedlings pre-steeped in cold manganese sulphate solution.

The values for ascorbic acid in seeds soaked for 24 hours in the cold and at room temperature were, respectively, 7·68 and 36·74 mgs. per cent. Hence, the effect of this pre-treatment on enhanced vitamin formation is observable only after germination has commenced.

The observations on the effect of cold treatment were extended to other leguminous seeds; those chosen were Peas, *Matki*, *Masöör* and Bengal gram (Table VIII).

TABLE VIII

*Effect of cold steeping on other legume seedlings*

| Day of generation | Untreated | Cold treated for 24 hours | Percent difference |
|---|---|---|---|
| | Ascorbic acid in mgms. per 100 gms. | | |
| | *Peas* (*Pisum arvense*) | | |
| 5 .. | 43·3 | 54·9 | +26·79 |
| 8 .. | 70·8 | 90·2 | +27·4 |
| | *Masöör* (*Lens esculenta*) | | |
| 5 .. | 168·7 | 221·2 | +31·13 |
| 8 .. | 227·2 | 256·8 | +13·03 |
| | *Matki* (*Phaseolus aconitifolius*) | | |
| 4 .. | 341·8 | 391·7 | +14·63 |
| 6 .. | 301·0 | 336·4 | +11·76 |
| | Bengal gram (*Cicer arietinum*) | | |
| 12 .. | 103·3 | 112·0 | + 8·42 |
| 15 .. | 126·7 | 119·8 | − 5·45 |

Sampling days were chosen taking into account the rate of growth of the seedlings which, as may be expected, varied with the different legumes, and were those when maximum ascorbic acid formation occurred.

With Bengal gram, where alone the effect was small, the studies were extended to seeds steeped in the cold for 48 hours. The ascorbic acid contents on the 12th and 15th days of sampling were, in order, 116·4 and 135·5 mgms. per 100 gms., corresponding to 12·69 and 6·87 per cent. respectively of increase over untreated seeds. Bengal gram, being rather slow to germinate on account of its large size and thicker seed coat, longer periods of cold steeping may possibly be necessary to give a definite increase.

The foregoing observations on the effect of cold treatment on ascorbic acid synthesis during germination were followed up by a study of other related changes, *viz.*, dehydroascorbic acid, and total ascorbic acid. The data on ascorbic acid oxidase changes are reported elsewhere (next paper).

In Table IX is given the day to day variations in ascorbic acid, dehydroascorbic acid and total ascorbic acid over a period of 9 days with pea seeds

TABLE IX

*Effect of cold treatment on the changes in total ascorbic acid in* Pea *seedlings*

| Day of germination | Ascorbic acid | | | Dehydro-ascorbic acid | | | Total ascorbic acid | | |
|---|---|---|---|---|---|---|---|---|---|
| | mgs. % | | Percent difference over untreated | mgs. % | | Percent difference over untreated | mgs. % | | Percent difference over treated |
| | untreated | treated | | untreated | treated | | untreated | treated | |
| 1 | 17·35 | 6·14 | −64·64 | nil | nil | nil | 17·35 | 6·14 | −64·64 |
| 2 | 25·23 | 20·54 | −14·62 | nil | nil | nil | 25·23 | 20·54 | −14·62 |
| 3 | 36·38 | 38·88 | + 6·87 | nil | nil | nil | 36·38 | 38·88 | + 6·87 |
| 4 | 37·59 | 42·36 | +12·69 | nil | nil | nil | 37·59 | 42·36 | +12·69 |
| 5 | 39·14 | 44·64 | +14·06 | 0·87 | 0·93 | .. | 40·01 | 45·57 | +13·96 |
| 6 | 40·01 | 48·03 | +20·04 | 3·68 | 1·24 | −60·87 | 43·69 | 49·27 | +12·77 |
| 7 | 41·58 | 51·12 | +22·95 | 6·14 | 1·81 | −70·52 | 47·72 | 52·93 | +11·45 |
| 8 | 48·73 | 59·77 | +22·66 | 7·61 | 3·02 | +60·32 | 56·34 | 62·79 | +11·45 |
| 9 | 61·72 | 73·19 | +18·58 | 8·81 | 4·10 | −53·46 | 70·53 | 77·29 | + 9·58 |

pretreated in the cold for 24 hours; corresponding values for untreated seeds are included.

Germinated seeds were not sampled for analysis beyond 9 days as the seedlings were no longer normal and healthy and were susceptible to fungal attack. It is interesting to note that while cold treatment is followed by an increase in ascorbic acid and in total ascorbic acid, the dehydroascorbic acid values are less; it is shown later that ascorbic acid oxidase activity also decreases as a result of this treatment (next paper).

The distribution of the vitamin in leaves, cotyledons and stalks of 5-day old *müng* seedlings grown from cold treated (24 hours) seeds is shown in Table X.

TABLE X

*Effect of cold treatment on the distribution of ascorbic acid in* Müng *seedlings*

| | Untreated | | Treated | | Percent difference in Total ascorbic acid |
|---|---|---|---|---|---|
| | Ascorbic acid | Dehydro-ascorbic acid | Ascorbic acid | Dehydro-ascorbic acid | |
| | (mgms. per 100 gms.) | | | | |
| Leaves .. | 491·5 | 14·3 | 543·10 | 14·5 | +10·24 |
| Cotyledons .. | 86·23 | 25·8 | 83·12 | 22·5 | − 5·81 |
| Stem .. | 125·8 | 21·5 | 114·40 | 18·4 | − 9·85 |

The main seat of biosynthesis of ascorbic acid lies in the leaves and any increase in ascorbic acid or total ascorbic acid as a result of cold treatment is therefore best observed with the leaves.

*Effect of germination in the dark*

*Müng* seeds, after overnight soaking in water, were germinated in an improvised chamber, cut off from all light. Detectable physical changes were absence of chlorophyll formation in the leaves and general yellowing of the entire seedlings. The stalk was juicy and less fibrous than in control seedlings germinated in diffuse light. Occasionally, with some of the seedlings grown in the dark, there was incidence of a peculiar infection; however, the data reported are for seedlings which were apparently healthy and for which any difference in regard to general conditions of growth was such as could only be attributed to absence of day light.

Data with seedlings sampled on 1st, 3rd and 5th days of germination are reported in Table XI. The vitamin values were in complete agreement by both the Harris and Olliver's[1] and Mapson's[2] procedures so that it can be taken that artefacts are excluded.

TABLE XI

*Effect of darkness on the formation of ascorbic acid by* Müng *seedlings*

| Day of germination | Per cent difference over untreated seedlings | | |
|---|---|---|---|
| | Ascorbic acid | Dehydro-ascorbic acid | Total ascorbic acid |
| 1 | nil | nil | nil |
| 3 | +69·00 | −55·38 | +47·63 |
| 5 | +32·00 | −39·07 | +21·19 |

It may be observed that, as with cold-treatment, there is marked increase in ascorbic acid and in total ascorbic acid as a result of germination in the dark but the dehydro-ascorbic acid values are less when compared with control seedlings.

The effect of germination in the dark was less pronounced with other seeds (*Pisum arvense*, *Lens esculenta* and *Cicer arietinum*), where, it must be stated, considerable difficulty was encountered in successfully germinating seed lots in the absence of light without microbial contamination. Chemicals

TABLE XII

*Effect of darkness on the distribution of ascorbic acid in* müng *seedlings*

| | Untreated | | Treated | | Percent difference in total ascorbic acid |
|---|---|---|---|---|---|
| | Ascorbic acid | Dehydro-ascorbic acid | Ascorbic acid | Dehydro-ascorbic acid | |
| | (Mgms. per 100 gms.) | | | | |
| Leaves .. | 491·5 | 14·3 | 604·4 | 16·0 | +22·66 |
| Cotyledons .. | 86·23 | 25·8 | 75·7 | 22·2 | −12·67 |
| Stem .. | 125·8 | 21·5 | 126·1 | 18·1 | − 2·51 |

like sodium benzoate, sulphur dioxide and formaldehyde were tried to check the growth of organisms but it was not considered desirable to extend these trials on account of their possible harmful effects on the germination process itself. $Mn^{++}$, again, showed no effect.

In Table XII is given the distribution of vitamin C in leaves, cotyledons and stalks of 5-day old seedlings grown in the dark and its comparison with those of corresponding seedlings grown in daylight.

It is significant that the favourable effect of germination in the dark on ascorbic acid formation is confined only to the region of the leaves and that there is a decrease in total ascorbic acid formation in the cotyledons and less so in the stem portion. It is interesting to speculate whether the absence of chlorophyll formation in the dark has anything to do with the extent of ascorbic acid elaboration.

### *Effect of cold treatment during steeping followed by germination in the dark*

In view of the pronounced effects brought about by germination in dark (Table XI) as well as by pretreatment by soaking at low temperatures, on the extent of biosynthesis of ascorbic acid (Table VII), it was thought worthwhile to study the combined effects of these two factors on ascorbic acid formation.

*Müng* seeds, steeped in water and kept inside a refrigerator for 6, 24 and 30 hours, were allowed to germinate in the dark. A second lot of seeds was germinated as usual in diffuse light after preliminary steeping overnight at room temperature while a third lot was allowed to germinate in the dark without preliminary cold treatment. Samples for analysis were taken on 5th day of germination (Table XIII).

TABLE XIII

*Effect of preliminary cold treatments, followed by germination in the dark*

| Untreated : normal steeping and germination | Germinated in the dark | Percent diff. | Cold steeped and germinated in dark | Period of steeping | Percent diff. over seeds germinated in dark | Percent diff. over untreated |
|---|---|---|---|---|---|---|
| 114·3 | 141·5 | +23·8 | 148·2 | 6 hrs. | + 4·74 | +29·66 |
| | | | 167·6 | 24 ,, | +18·45 | +43·63 |
| | | | 173·4 | 30 ,, | +22·54 | +51·70 |

Cold treatment followed by germination in the dark resulted in greater formation of ascorbic acid than germination in the dark alone.

*Effect of exposure to ultra-violet rays*

The effect of irradiation on ascorbic acid synthesis was studied in two ways. In one set of experiments (I), seeds, soaked overnight in water, were spread out on moistened cotton wads in Petri dishes and then exposed to ultra-violet rays from a Hanovia unit for a period of 30 minutes. The source of light was adjusted to a distance of 12 inches from the surface of the exposed dishes which were kept vertically underneath the lamp unit. The irradiated seeds were then allowed to germinate as usual.

The second set (II) of experiments was carried out as above except that irradiation was given for a period of 10 minutes on the 1st day of germination and on every subsequent day after the usual waterings.

Samples were taken for ascorbic acid determination on the 3rd and 5th days of germination together with corresponding samples from seedlings simultaneously grown without any ultra-violet treatment. Results are given in Table XIV.

TABLE XIV

*Effect of irradiation on* Müng *seedlings*

| Day of germination | Untreated | Ultra-violet ray treated | |
|---|---|---|---|
| | | I | II |
| | Ascorbic acid in mgms. per 100 gms. | | |
| 3 .. | 152·3 | 155·9 | 146·2 |
| 5 .. | 178·5 | 185·3 | 163·5 |

Irradiation of soaked seeds has practically no effect while daily irradiation seems to have affected germination adversely.

*Effect of seed treatment with plant hormones*

Seeds (*Müng* and *Masöör*) were soaked overnight in 50 p.p.m. solutions of α-napthyl acetic acid and β-indolyl acetic acid (B.D.H.), and then allowed to germinate as usual; subsequent waterings during germination were done only with distilled water. The hormone solutions were prepared by dissolving weighed amounts in one or two drops of absolute alcohol and then dilut-

ing to calculated volumes. Seedlings were sampled on 5th and 8th days. Results are given in Table XV together with values for untreated seedlings.

It was observed that hormone treatment resulted in stunted seedlings which had bunched short roots, several in number. This effect was striking in the case of α-napthyl acetic acid. There was a detrimental effect on ascorbic acid formation during germination which was more pronounced in the initial stages and which tended to diminish or disappear subsequently.

TABLE XV

*Effect of hormone pre-treatment*

| Day of germination | Untreated | α-naphthyl acetic acid | Percent diff. | β-Indolyl acetic acid | Percent diff. |
|---|---|---|---|---|---|
| | Ascorbic acid in mgms. per 100 gms. | | | | |
| | *Müng* (*Phaseolus radiatus*) | | | | |
| 5 .. | 179·2 | 89·5 | −50·05 | 143·8 | −19·75 |
| 8 .. | 211·0 | 217·4 | + 3·03 | 208·8 | − 1·04 |
| | *Masöör* (*Lens esculenta*) | | | | |
| 5 .. | 143·5 | 85·3 | −40·56 | 137·3 | −4·32 |
| 8 .. | 244·0 | 179·1 | −26·60 | 250·2 | +2·48 |

*Effect of Colchicine.—Müng* seeds* were soaked overnight in a 50 p.p.m. solution of colchicine and then germinated as usual. Also, tetraploid *müng* seeds, harvested from plants grown after colchicine treatment of diploid seeds, were simultaneously germinated under normal conditions. Samples for analysis were taken on 3rd and 5th days together with control lots (Table XVI).

TABLE XVI

*Effect of seed treatment with colchicine*

| Day of germination | Untreated | Colchicine treated | Percent diff. over untreated | Tetraploid | Percent diff. over untreated |
|---|---|---|---|---|---|
| | Ascorbic acid in mgms. per 100 gms. | | | | |
| 3 .. | 152·3 | 118·2 | −22·40 | 123·9 | −18·65 |
| 5 | 178·3 | 179·1 | + 0·45 | 170·2 | − 4·54 |

* Samples of the diploid and tetraploid *müng* seeds were obtained through the courtesy of Principal L. S. S. Kumar, *College of Agriculture, Poona*.

It was noted that tetraploid seeds gave seedlings with stunted growths; leaves were deeper green in colour than those from untreated seeds. As with hormone, both colchicine treated seeds and tetraploid seeds gave lesser values for ascorbic acid on germination when sampled on the 3rd day while on the 5th day of germination more or less identical values were obtained.

In the experiments on the effects of exposure to ultra-violet rays, colchicine treatment and polyploidy, the seedlings withered on the 6th day and hence further samplings could not be done.

### *Effects of sugars*

A different technique was adopted for germinating the seeds. The seeds (*müng*) were soaked for 6 hours in sterile distilled water and the embryos, separated from the cotyledons, were placed under sterile conditions on a nutrient medium soft enough to just allow radicals to pierce through. Knopp's solution was used to provide nutrients for the growth of the embryo. Ray[6] had recommended the use of 10 per cent. gelatin for the preparation of semi-solid nutrient media but under the desiccating atmospheric conditions prevailing at the time of these experiments, the medium so prepared soon dried to a thin solid film; by lowering the concentration of gelatin or using agar agar, this drying could be minimised. The best concentration was found to be 0·4 per cent. agar agar. The semi-solid gel, set in a Petri dish, was covered with a thin layer of 100 p.p.m. sodium benzoate solution in water, after the excised embryos were transplanted to it, and was placed inside a sterile humid chamber in order to keep the nutrient gel from drying. The appropriate sugar was dissolved in the semi-solid medium in a 5 per cent. concentration.

TABLE XVII

*Effect of sugars on ascorbic acid synthesis by embryos*

| Treatment | Ascorbic acid mgms. per 100 gms. | Percent difference over untreated |
|---|---|---|
| Nil .. | 60·3 | .. |
| Glucose .. | 131·3 | +117·8 |
| Mannose .. | 156·8 | +160·1 |
| Sucrose .. | 75·0 | + 24·4 |

Samples were taken for ascorbic acid determination on 5th day of germination together with corresponding samples from embryos simultaneously grown without any sugar addition. A typical set of results, obtained by Mapson's procedure, is given in Table XVII.

Attempts were made to grow the embryos on cotton wads soaked with the nutrient medium but were later abandoned as the moistured cotton wad was more difficult to be pierced through by the radical than the semi-solid gel.

The results would support the hypothesis that ascorbic acid is probably a product of carbohydrate metabolism.

*Synthesis by micro-organisms*

An attempt was made to study the effects of two strains of *Aspergillus niger*, producing citric acid and gluconic acid respectively, and one of *B. prodigiosus* on Vitamin C formation from sugars in culture media (*Cf.* 7, 8).

Several tubes with 5 c.c. of culture medium[9] with the different sugars in 10 per cent. concentration, were inoculated with water suspensions of the two moulds from potent strains obtained by three subculturings from stock cultures. Sugars used for the purpose were mannose, sucrose and fructose and were added after they were separately autoclaved. These, together with control uninoculated ones, were kept at room temperature (28° C.); no ascorbic acid could be detected on any day, during 12 days, in the inoculated as well as in the blank tubes.

Similar trials with *B. prodigiosus* inoculated into nutrient broth containing xylose or the above sugars in 10 per cent. concentrations also yielded negative results. However, it must be stated here that, when the nutrient broth was autoclaved together with the sugar dissolved in it, production of large quantities of artefacts had occurred.

## Discussion

$Mn^{++}$ has been claimed to stimulate ascorbic acid formation in plants[10, 11, 12] as well as in germinating seeds.[4, 13, 14] The studies reported here show, on the other hand, that, over a range of concentrations, $Mn^{++}$ has failed to influence biosynthesis of ascorbic acid in germinating seedlings (*Cf.* 15, 16) and hence it is doubtful if $Mn^{++}$ acts as coenzyme in dehydrogenase systems responsible for ascorbic acid synthesis.[17]

Contrary to the claims of Shen *et al.*,[3] $Mg^{++}$, like $Mn^{++}$, also has little effect, but treatment with boric acid seems to influence to some extent ascorbic acid formation in seedlings and would warrant extended study. Earlier, Harris[11] and Ahmed *et al.*[4] had observed a similar stimulating effect due to boron treatment, but Lyon *et al.*[15, 16] could not obtain any increase due to boron treatment, and hence did not share this view.

Though ascorbic acid is not a product, directly, of photochemical action, light is held to help its synthesis in the growing plant. The concentration of ascorbic acid in plants is stated to be a function of light received, being maximum in the afternoon.[17] Correlation between chlorophyl content and extent of ascorbic acid synthesis has also been suggested,[14, 19, 20] but the indications from the data reported here are otherwise. A large increase in ascorbic acid content of seedlings germinated in dark over those grown normally has been shown here. In spite of the increased values obtained for ascorbic acid or for total ascorbic acid as a result of germination in the dark, there is a distinct fall in dehydroascorbic acid.

The high total ascorbic acid and low dehydroascorbic acid values as a result of germination in the dark may be due to the absence of photo-oxidation. That ultra-violet rays catalyse the oxidation of ascorbic acid is well known; also, this is borne out by the fact that when seedlings were exposed to ultra-violet irradiation for 10 minutes daily, a decrease in ascorbic acid content was observable (Table XIV).

There is therefore reason to believe that a considerable portion of ascorbic acid might have been oxidised to dehydroascorbic acid as well as, irreversibly, in light. It is also possible, although it cannot be stated on the basis of the results here, that the reducing mechanism, presumably the dehydroascorbic acid reductase[21, 22] which is responsible for the reduction of dehydroascorbic acid to ascorbic acid, functions better in the absence of light.

In experiments on the growth of embryos in nutrient media, addition of sugars results in increased formation of ascorbic acid, the monosaccharides being more effective than the disaccharides; the small increase in ascorbic acid with sucrose treatment might possibly be due to its partial inversion. Sugars, chiefly monosaccharides, may play the role of precursors in the biosynthesis of ascorbic acid, but as yet there is no understanding of the mechanism involved. This is further discussed in the next communication.

Ahmed *et al.*[4] found significant stimulating effect on biosynthesis of vitamin C by germinating embryos as a result of addition of α-chlorophyll.

They also observed increased ascorbic acid formation in seedlings germinated in light (shade and direct sunlight) than those germinated in the dark. Presumably, according to them, the high content of the vitamin in seedlings grown in light was related to chlorophyll formation. But seedlings germinated in the dark were completely devoid of chlorophyll. Further, germinating tetraploid seeds, judging from the colour of their foliage, contained more chlorophyll than the leaves of control diploid seeds. The findings reported here are in disagreement with those of Ahmed on the effects of darkness and of chlorophyll on ascorbic acid synthesis by germinating seeds; the case of growing plants must, it is recognised, be regarded as totally different, since here chlorophyll plays an important part in the photosynthesis of sugars, whereas in germinating seeds these are formed through hydrolysis of the stored carbohydrates.

As significant as the observations on the effect of darkness are those relating to the effect of pre-treatment by steeping in the cold. The changes in ascorbic acid and total ascorbic acid on the one hand and in dehydroascorbic acid on the other, are similar in both cases. As, however, there has been no modification in environmental conditions such as would affect possible photo-oxidative changes in ascorbic acid that has been assumed in explaining the effect of germination in the dark, a different mechanism must have been responsible for this effect. It is interesting that the effects of darkness and cold treatment during steeping are partly additive.

The studies on the distribution of ascorbic acid in the various parts of the seedlings have shown that the leaf is richest in the vitamin and that therefore the changes due to germination in the dark as well as after cold treatment are most shown in the leaf. But, it is seen that though dehydroascorbic acid in leaves increased slightly due to the two treatments, that of the treated seedlings as a whole, is significantly less. This is to be expected since leaves and cotyledons (in 5-day old seedlings) constitute only less than 5 and 10 per cent. respectively of the whole seedlings; on quantitative basis, the stems form the major part of the seedlings, and would largely govern the extent of the effect of any treatment of the whole seedling.

Though Roy, Bose and Guha[23] have reported that irradiation of germinating soy beans and *kancha müng* by ultra-violet light results in the accumulation of indophenol reducing substances which were different from ascorbic acid, it is observed here that irradiation has, if anything, resulted in a slight retardation in ascorbic acid synthesis. The decreased ascorbic acid formation in seeds exposed daily to ultra-violet light is to be expected, since ascorbic acid oxidation is catalysed *in vitro* by ultra-violet rays.

Hormone and colchicine treatments have shown an initial depressing effect which later diminished. More extended trials seem necessary before their effects can be definitely assessed.

In determining the effect of the salts of potassium, nitrogen and phosphorus, their concentrations were adopted from those followed by Mapson and Cruickshank,[5] but these were found to be rather drastic. As has been emphasised[15, 24] ammonium sulphate has a greater depressing effect on ascorbic acid synthesis than potassium nitrate, when the same quantity of nitrogen is supplied in the form of these two salts. The former also inhibits the growth of the seedlings to a greater extent than the latter, but the stimulation of ascorbic acid synthesis, stated to be the case by Mapson and Cruickshank (*loc cit.*), is not observable; in fact, germinating seeds, treated with potassium nitrate, show pronounced decrease over the corresponding untreated seedlings. The depressing effect of the urea treatment in the same molecular proportion as ammonium sulphate, both on growth of the seedling as well as on ascorbic acid formation is markedly less as compared to the effects of the other two inorganic nitrogen compounds; on the 5th day, ascorbic acid formation in urea treated seedlings is nearly equal to that in untreated seedlings.

Phosphate treatment, as also the combined use of nitrogen, phosphorus and potassium salts have retarded growth and reduced ascorbic acid formation, the effects diminishing with the progress of germination. The similarity with the effects of hormone and colchicine treatments is apparent. In assessing the effects of nutrients and stimulants, the doses for treatment should be chosen so as not to hamper growth since this has an obvious bearing on ascorbic acid synthesis.

The studies on polyploid seeds (*müng*) have also shown a decreased ascorbic acid formation, specially during the early stages of germination which later tend to equalise with the ascorbic acid levels in untreated seeds. Sansome and Zilva[25] reported that, with the tomato fruit, all tetraploid strains were about twice as rich in ascorbic acid as diploids. A similar observation with apple was made by Crane and Zilva[26] earlier, though these results were not in agreement with Key's[27] findings on tomato, where there was no increase in ascorbic acid on account of polyploidy. It cannot however be concluded that because no increased ascorbic acid formation could be observed during germination, the fruits of the polyploid seeds would behave similarly.

The studies on the synthesis of vitamin C by the two micro-organisms have yielded negative results, but the possibilities of such a synthesis by

specific strains of certain organisms cannot be ignored; the production of artefacts is an important factor here that may lead to erroneous results.

Finally, the observation that during the ripening of fruits the proportion of dehydroascorbic acid to ascorbic acid is maximum in the raw fruit and that this ratio decreases with the maturity of the fruit[28, 29, 30] has been taken to suggest that dehydroascorbic acid is a possible immediate precursor of ascorbic acid. However, in germinating seeds, while the contents of both ascorbic acid and dehydroascorbic acid rise, the ratio of the former to the latter decreases with the period of germination. If dehydroascorbic acid was the immediate precursor of ascorbic acid in germinating seeds, the proportion of the former to the latter would have been maximum in the overnight soaked seeds, but since it is the least in such seeds, it would mean that the presence of dehydroascorbic acid in the seeds during further germination is on account of the *in vivo* oxidation of ascorbic acid, and hence dehydroascorbic acid cannot be considered as the immediate precursor of ascorbic acid, at any rate, in germinating seeds. Even in the fruits, Rangnekar[30] had observed that there was present, in the very raw stage, a powerful oxidase which decreased in its activity with maturity of fruit; this shows that the rise or fall in the proportion of dehydroascorbic acid of the fruit parallels oxidase activity and that therefore dehydroascorbic acid arises from the oxidation of ascorbic acid. Further evidence in respect of this possibility is presented in the next communication.

## Summary

The effects of various cultural and environmental factors on the biosynthesis of ascorbic acid in germinating seeds have been studied. It is observed that treatments with manganese ions, over a range concentrations, and in different ways, are ineffective in bringing about any change in ascorbic acid or dehydroascorbic acid formation, or in the distribution of these constituents in the various parts of the seedling. Similar results are obtained with magnesium ion, while boric acid treatment slightly enchances ascorbic acid synthesis.

Treatments with different nitrogenous, phosphatic and potassic fertilizers, plant hormones, and colchicine, result in an inhibition of growth accompanied by a pronounced decrease in ascorbic acid formation in the earlier stages of germination. The extent of biosynthesis of vitamin C during germination of polyploid seeds is similar to that in hormone or colchicine treated seeds. Exposure of seedlings to ultraviolet rays is without significant effect on the synthesis of the vitamin.

Among other treatments studied, steeping of seed in the cold, and germination in the dark, effect pronounced increases in the biosynthesis of ascorbic acid, the latter being relatively more effective. Also, a cold treatment of the seed followed by germination in the dark results in greater formation of ascorbic acid than germination in the dark alone. With cold treatment, it is seen that the degree of effectiveness depends, within limit, on the duration of the treatment.

Addition of hexoses to nutrient media increases two-fold ascorbic acid formation in seed embryos; attempts to elaborate ascorbic acid from sugars through the growth of certain micro-organisms have not however been successful.

The observations are discussed in relation to the possible changes involved in the mechanism of the biogenesis of ascorbic acid in germinating seeds.

## References


1. Harris and Olliver .. *Biochem. Jour.*, 1942, **36**, 1061.
2. Mapson .. *Jour. Soc. Chem. Ind.*, 1943, **62**, 226.
3. Shen, Hsieh and Chen .. *Biochem. Jour.*, 1945, **39**, 107.
4. Ahmed, Quereshi, Babbar and Sawhney .. *Ann. Biochem. Expt. Med.*, 1946, **6**, 29.
5. Mapson and Cruickshank .. *Biochem. Jour.*, 1947, **41**, 197
6. Ray .. *Ibid.*, 1934, **28**, 996.
7. Fukumoto and Shimomura .. *Jour. Agri. Chem. Soc.*, (Japan,) 1937, **13**, 613.
8. Busing and Peters .. *Biochem. Z.*, 1940, **304**, 134.
9. Karrow and Waksman .. *Ind. Eng. Chem.*, 1947, **39**, 821.
10. Hester .. *Science*, 1941, **93**, 401 ; *Proc. Soil Sci. Soc. Am.*, 1941, **6**, 243.
11. Harris .. *Proc. Am. Soc. Hort. Sci.*, 1944, **44**, 300.
12. Rangnekar .. *Curr. Sci.*, 1945, **14**, 325.
13. Rudra .. *Nature*, 1938, **141**, 203.
14. ——— .. *Ibid.*, 1944, **153**, 111, 743.
15. Lyon and Beeson .. *Jour. Am. Soc. Agron.*, 1943, **35**, 166.

    ——— ,——— and Ellis .. *Botan. Gaz.*, 1943, **104**, 495.
16. Lyon and Parkes .. *Ibid.*, 1944, **105**, 392.
17. Rudra .. *Nature*, 1943, **151**, 641.
18. Smith and Gillies .. *Biochem. Jour.*, 1940, **34**, 1312.
19. Giroud, Ratsimamanga and Leblond .. *Compt. Rend. Soc. Biol.*, 1935, **118**, 874.

20. Randoin, Giroud and Leblond .. *Compt. Rend. Soc. Biol.*, 1935, **120**, 297.
21. Crook .. *Biochem. Jour.*, 1941, **35**, 226.
22. ——— and Morgan .. *Ibid.*, 1944, **38**, 10.
23. Roy, Bose and Guha .. *Science and Culture*, 1944, **9**, 564.
24. Virtanen and Hausen .. *Acta. Chem. Fennicae*, 1932, **B 5**, 45.
25. Sansome and Zilva .. *Biochem. Jour.*, 1933, **27**, 1935.
26. Crane and Zilva .. *Jour. Pomolgy and Hort. Sci.*, 1939, **131**, 57.
27. Key .. *Biochem. Jour.*, 1933, **27**, 153.
28. Zilva, Kidd and West .. *New Phytol.*, 1938, **37**, 345.
29. West and Zilva .. *Biochem. Jour.*, 1944, **38**, 105.
30. Ranganekar .. *Proc. 33rd Indian Sci. Congr.*, Part III, 1946, 41.

# STUDIES IN THE EMBRYOLOGY OF SOME MEMBERS OF THE BIGNONIACEÆ*

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## Introduction

Several genera of the Bignoniaceæ occur in India and elsewhere, but the embryology of only a few of these has been studied. The most important recent work is by Mauritzon (1935), who recognised two main types of endosperm development, *viz.*, the *Catalpa* type and the *Incarvillea* type. In India, Swamy (1941) studied megasporogenesis and endosperm formation in *Bignonia megapotamica* and Venkatasubban (1944, 1945) has worked on the cytology of some members of the family. The present paper gives an account of megasporogenesis and endosperm formation in *Jacaranda mimosæfolia* D. Don, *Parmentiera cerifera* Seem., and *Tecoma stans* (L) H.B.K. Development of the female gametophyte of *Kigelia pinnata* DC. is also included. All the four plants are cultivated in local horticultural gardens.

## Materials and Methods

For microscopic studies, the material was fixed in Allen's modification of Bouin's fluid. Microtome sections 10–26 $\mu$ thick were cut and stained in Heidenhain's Iron-alum Hæmatoxylin with eosin in clove oil as counterstain, or in Newton's iodine gentian violet.

## Organogeny

The development of the floral parts could be studied only in *Tecoma stans*. The floral primordium takes its origin in the axil of a bract (Figs. 55, 56) and differentiates into an upper floral receptacle and a lower pedicel.

The axis of the inflorescence is triangular in cross-section (Fig. 62). The florets arise successively at the three corners of the axis in an anticlockwise direction (Figs. 62–66). In the floral primordium, the individual parts are differentiated in acropetal succession; calyx, corolla, stamens and carpels (Figs. 55–61).

* Part of the thesis submitted to the University of Mysore in partial fulfilment of the requirements of the degree of Master of Science in 1948.

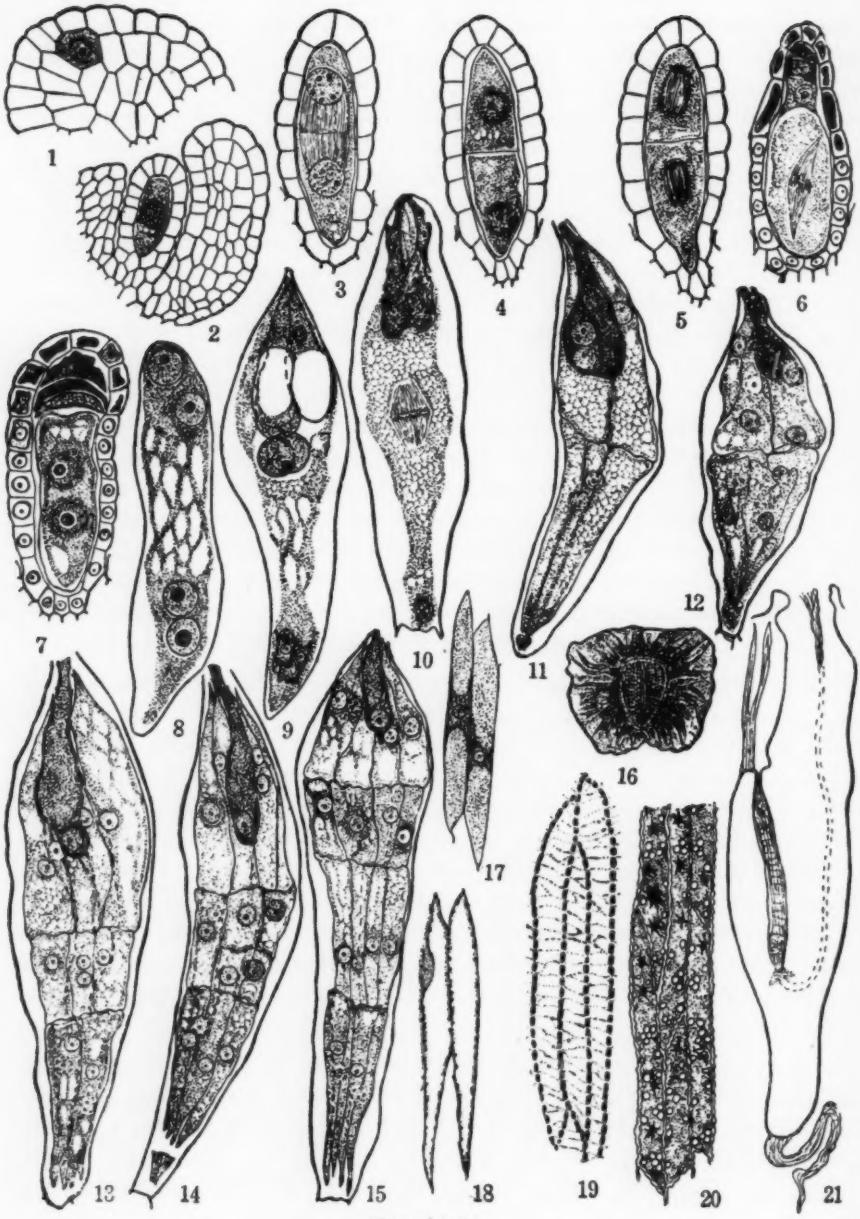
After the formation of the bract (Figs. 67–70) on the anterior side, the two bracteoles are differentiated laterally (Figs. 71–75). The primordium of the calyx first arises as a complete ring (Figs. 76–81). Its two antero-lateral lobes are the first to differentiate (Figs. 85–86) followed by the other three which arise almost simultaneously (Figs. 87–88). The sepals show a valvate æstivation. In the development of the corolla the single anterior lobe arises first (Figs. 81, 82), then the two antero-lateral lobes (Figs. 82–84), and finally the two postero-laterals (Figs. 83–85). They show an imbricate æstivation with the anterior petal innermost (Fig. 84). Of the stamens the two anterior ones are formed first (Fig. 80) and then the two posterior laterals (Figs. 81, 82). The staminode is posterior (Fig. 84). Of the two carpels the anterior (Fig. 80) precedes the posterior in development (Figs. 81, 82).

## Megasporangium and Megasporogenesis

In all the four genera the ovary is superior, subsessile, bicarpellary and bilocular with a single style and a bilobed flattened stigma. It is surrounded by a thick annular honey secreting disc. There is an indefinite number of unitegmic anatropous ovules, borne on the slightly projecting axile placenta. The ovules are uniformly distributed on the placenta in each locule in *Jacaranda* but form two groups in *Kigelia*, *Parmentiera* and *Tecoma*. While the ovules are usually anatropous, occasional occurrence of hemitropous ovules has been noted in *Kigelia* and *Parmentiera*.

The nucellar primordium is at first made up of a homogeneous mass of parenchymatous cells. The hypodermal archesporium is formed at an early stage becoming distinguishable from the surrounding cells even before the origin of the integuments (Figs. 1, 22, 34). It directly functions as the megaspore mother cell without cutting off any parietal cells (Figs. 2, 24, 35). Instances of the occurrence of a two-celled archesporium were met with only in *Kigelia* and *Tecoma* (Figs. 23, 89). At this stage the integument is two to four layers of cells in thickness. In *Parmentiera* (Fig. 35) it encloses the nucellus almost completely leaving only small narrow micropyle. In *Jacaranda* and *Tecoma*, however, at this stage the integument extends only to half the length of the nucellus, while in *Kigelia* (Fig. 24) it remains at a still lower level.

In all the plants under study the megaspore mother cell enlarges in size and undergoes the usual reduction divisions to form a linear tetrad of megaspores (Figs. 3–5, 25, 36, 37, 90–93). T-shaped tetrad were noticed in *Kigelia* (Fig. 28) together with one case showing an isobilateral arrangement of the megaspores (Fig. 27). Instances of this kind are rather rare in angiosperms and have been recorded in *Myrtus communis*, *Urginia indica* and *Daphne*




FIGS. 1–21

FIGS. 1-21. *Jacaranda mimosaefolia.*—Fig. 1. Nucellus showing archesporial cell × 679. Fig. 2. Megaspore mother cell × 485. Fig. 3. Formation of dyad × 970. Fig. 4. Dyad × 970. Fig. 5. Second meiotic division in dyad cells × 970. Fig. 6. Division of nucleus of functioning megaspore × 970. Fig. 7. Two nucleate embryo sac × 970. Fig. 8. Four nucleate embryo sac × 970. Fig. 9. Mature embryo sac × 679. Fig. 10. Primary endosperm nucleus in division × 679. Figs. 11–15. Stages in the development of the endosperm. Fig. 11. × 970 ; others × 679. Fig. 16. Winged seed × Nat. size. Figs. 17-20. Cells of the wing at different stages × 295. Fig. 21. Diagram showing chalazal strand, nutritive tissue and the four regions of the endosperm ; note the development of wings at the micropylar and the chalazal ends respectively × 50.

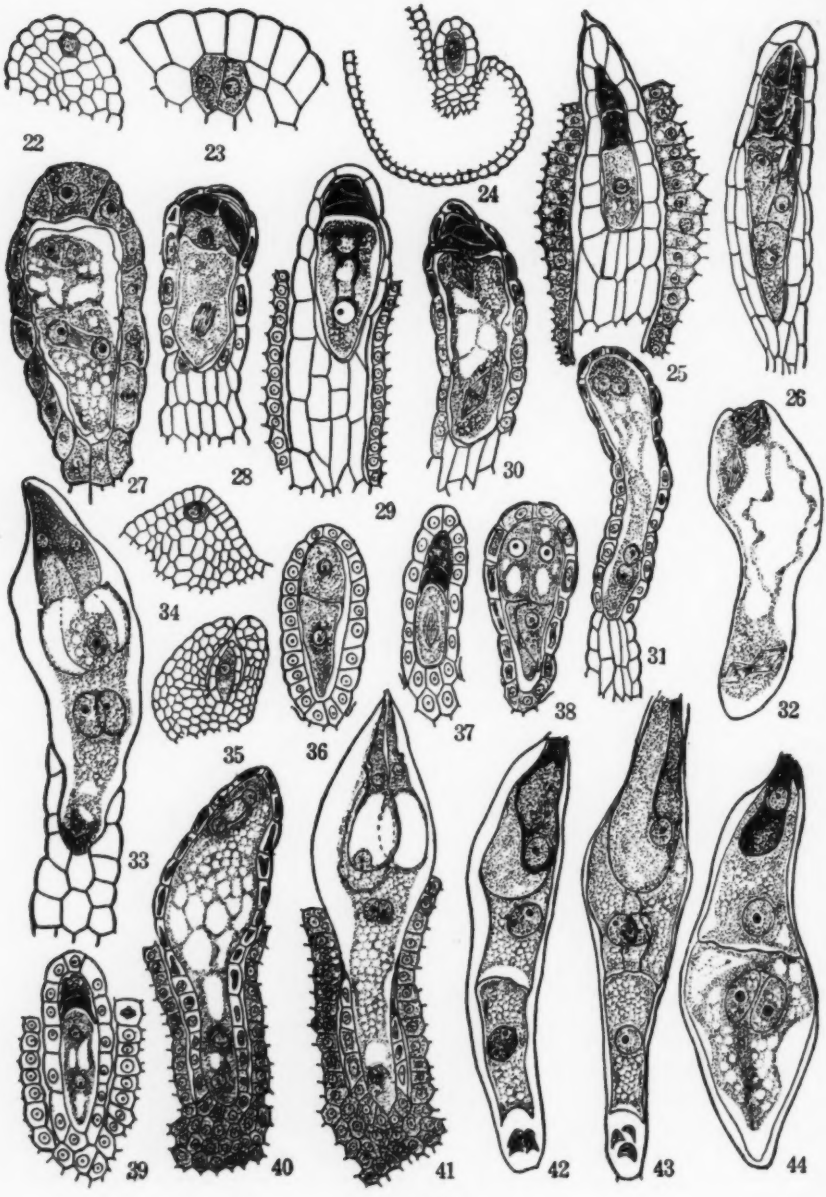
*laureola* (Maheshwari, 1937, 1941). One instance of a decussate arrangement of the megaspores was found in *Paramentiera* (Fig. 38).

The chalazal megaspore usually functions though sometimes the third megaspore of the tetrad may show signs of enlargement as in *Parmentiera*. Occasionally two linear tetrads were seen lying side by side in *Kigelia* (Fig. 26). In *Bignonia megapotamica* Swamy (1941) records the occurrence of double tetrads.

In all the plants studied, the ovule is tenuinucellate. At the micropylar end the nucellar epidermis is destroyed by the elongation of the embryo sac, but towards the chalazal end the nucellus persists up to the early stages of endosperm development. This feature is very clearly seen in *Paramentiera* and *Tecoma* (Figs. 41, 96) and has also been reported by Mauritzon (1935) in some members of the Bignoniaceæ studied by him. After the disorganisation of the nucellar epidermis, the innermost layer of the integument forms the tapetum, with radially elongated cells. This lies direct in contact with the developing embryo sac and reaches its maximum development at the time of the organisation of the embryo sac, being especially conspicuous around the non-dilated lower region of the sac (Figs. 41, 96).

The nucellus of the chalazal megaspore divides (Figs. 6, 28, 37) and the resulting nuclei separate towards the two poles (Figs. 7, 29, 39, 94) where they divide twice (Figs. 8, 30–32, 40, 95) to produce an eight-nucleate embryo sac of the *Polygonum* type (Maheshwari, 1948). During this process, the lower end of the embryo sac becomes somewhat elongated and penetrates into the chalazal region of the ovule. This is very clearly seen in *Parmentiera* (Fig. 40).

The mature embryo sac is dilated at the micropylar end, and tapers towards the chalazal end (Figs. 9, 41, 96). In *Kigelia* (Fig. 33) it is almost equally wide at both ends. The synergids are long and hooked and have pointed apices (Figs. 9, 33, 41) with the nucleus situated in this region. A similar condition is known in *Catalpa* (Mauritzon, 1935) and *Bignonia megapotamica* (Swamy, 1941). The polar nuclei are of approximately the same




FIGS. 22–44

FIGS. 22-33. *Kigelia pinnata*.—Fig. 22. Nucellus showing primary archesporial cell × 400. Fig. 23. Two-celled archesporium × 970. Fig. 24. Megaspore mother cell × 291. Fig. 25. Linear tetrad showing the three degenerating megaspores and the enlarging chalazal megaspore × 679. Fig. 26. Two linear tetrads lying side by side × 679. Fig. 27. Second meiotic division in dyad cells. The orientation of the spindles suggests the formation of an isobilateral tetrad × 970. Fig. 28. T-shaped tetrad ; chalazal megaspore dividing × 679. Fig. 29. Two-nucleate embryo sac with degenerating micropylar megaspores × 679. Fig. 30. Two-nucleate embryo sac in division × 679. Fig. 31. Four-nucleate embryo sac × 679. Fig. 32. Four-nucleate embryo sac in division × 679. Fig. 33. Mature embryo sac ; note degenerating antipodals × 679.

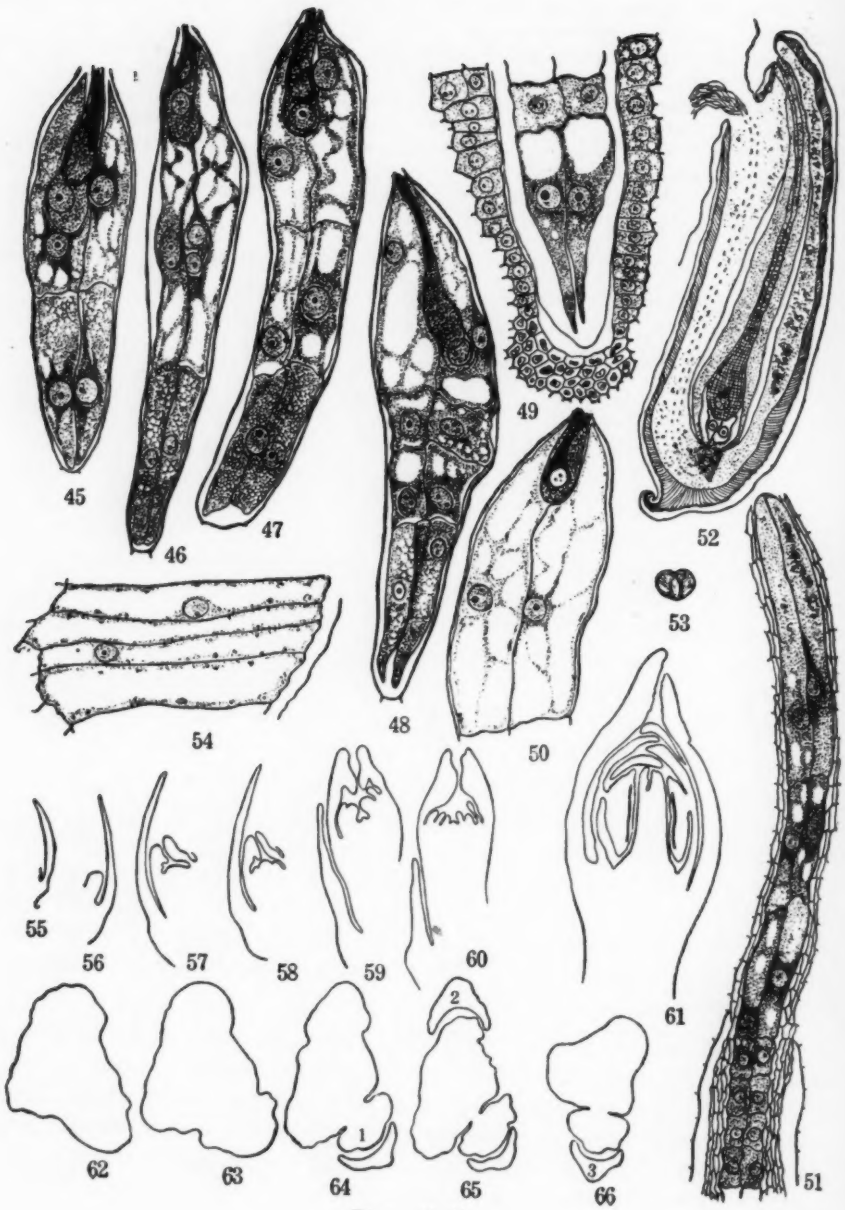
size and fuse prior to fertilization to form the secondary nucleus. The antipodals are organised as definite cells (Figs. 9, 33, 41, 96). In *Tecoma* (Fig. 96) the two lateral antipodal cells show basal vacuoles. In all the forms studied, except *Kigelia*, the antipodals persist up to the early stages of endosperm development (Figs. 10–12, 42, 43, 97, 99–101, 104). During the entry of the pollen tube into the embryo sac, one of the synergids is destroyed (Figs. 10, 97, 99). Double fertilisation occurs (Fig. 97).

## ENDOSPERM

The primary endosperm nucleus divides earlier than the egg. The first division gives rise to a large primary micropylar and a small primary chalazal chamber (*cf. Catalpa bignonioides*, Mauritzon, 1935). The next division is vertical in both chambers, resulting in the formation of two tiers of two cells each. In *Jacaranda* these two vertical divisions are almost synchronous (Fig. 11). In *Parmentiera*, either the primary micropylar chamber (Fig. 43) or the primary chalazal chamber (Fig. 44) may divide first. In *Tecoma* the primary micropylar chamber is usually the first to divide (Fig. 100).

Mauritzon (1935) distinguishes two main types of endosperm development in the Bignoniaceæ, the *Catalpa* type and the *Incarvillea* type. In the *Catalpa* type the micropylar and the chalazal haustoria are each four-celled. In the *Incarvillea* type both the haustoria are one-celled. In *Jacaranda*, *Parmentiera* and *Tecoma* the early development follows the *Catalpa* type with only a few minor variations. As the subsequent course of development, after the organisation of four-celled endosperm, is different in these plants, they are described separately.

In *Jacaranda* after the four-celled stage, vertical walls are laid down in the two primary tiers at right angles to the previous plane of division resulting in the formation of eight cells (Fig. 12). The four cells of the chalazal tier usually do not divide further, but elongate to form the chalazal haustorium. The four cells of the micropylar tier appear to divide transversely to form eight cells, so that the endosperm is now made up of three tiers, each consisting of four cells (Fig. 13). It is usually the middle tier of four cells which




FIGS. 45–66

FIGS. 34–54. *Parmentiera cerifera.*—Fig. 34. Nucellus showing archesporial cell × 400. Fig. 35. Megaspore mother cell × 485. Fig. 36. Dyad × 679. Fig. 37. Linear tetrad with nucleus of functioning megaspore in division × 679. Fig. 38. Decussate arrangement of megaspores × 970. Fig. 39. Two-nucleate embryo sac × 679. Fig. 40. Four-nucleate embryo sac ; note the persisting spindles at the micropylar end × 679. Fig. 41. Mature embryo sac ; note the integumentary tapetum lining the non-dilated chalazal portion of the embryo sac × 679. Fig. 42–48. Stages in the development of the endosperm. Figs. 42-47 × 970. Fig. 48. × 679. Fig. 49. Two-celled chalaval haustorium × 970. Fig. 50. Two cells of the weakly developed micropylar haustorium × 679. Fig. 51. Later stage showing micropylar haustorium and some endosperm cells × 200. Fig. 52. Diagram showing nutritive tissue, conducting strand and the four regions of the endosperm ; note the development of the wings × 70. Fig. 53. Winged seed × 2. Fig. 54. A few cells of the wing enlarged × 485.

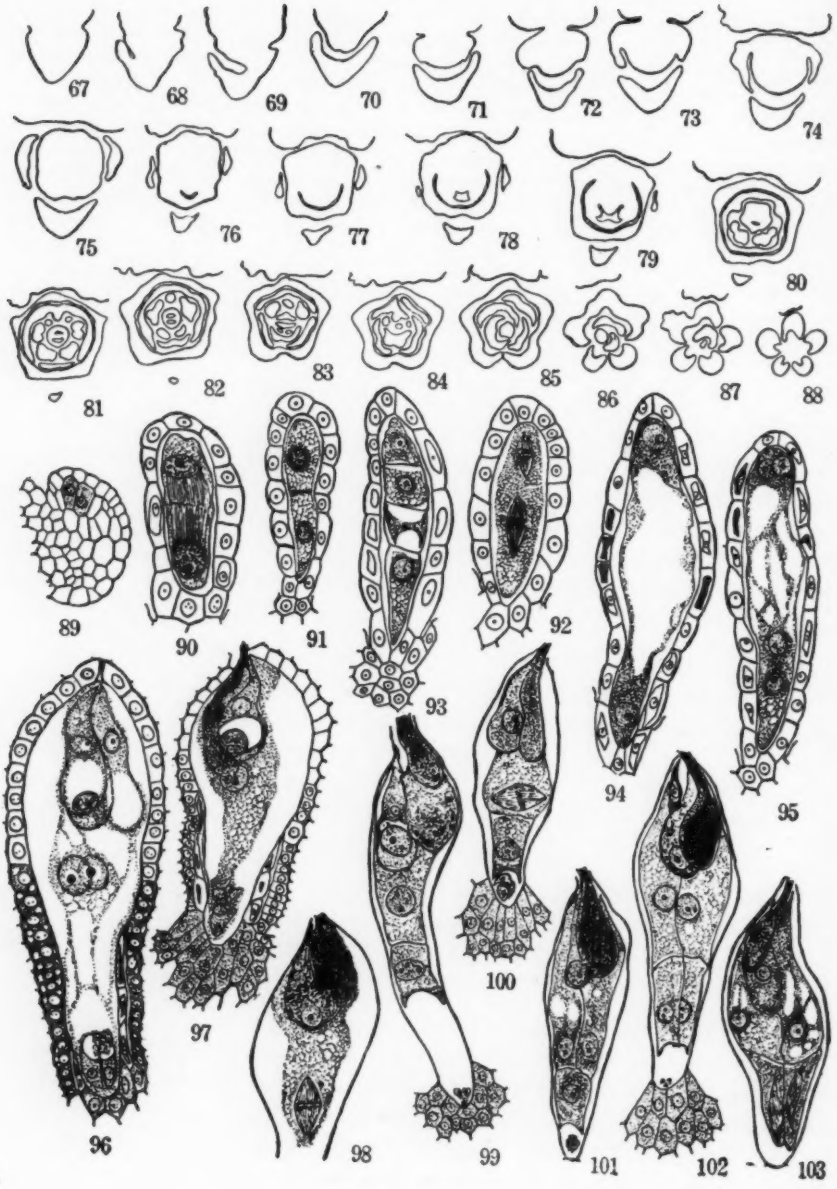
produces the endosperm proper (Fig. 14). The four cells of the chalazal haustorium have dense cytoplasm and because of the aggressive nature the cells in this part of the ovule become collapsed and present a degenerated appearance.

The four micropylar endosperm cells do not show such dense cytoplasm and probably do not have any significant haustorial function (Fig. 15). Mauritzon (1935) describes a similar type of development in *Catalpa bignonioides* and considers that in the formation of the endosperm tissue, the four micropylar cells have the role of mother cells cutting off cells at the lower end. The cells ultimately left at the top may remain merely as endosperm cells or function as weakly developed haustoria.

In *Parmentiera*, after the four-celled stage, the two cells of the lower tier become conspicuous and form the chalazal haustorium (Fig. 45). The two cells of the upper tier, divide (Fig. 46) transversely so that a six-celled endosperm is formed, made up of three tiers of two cells each (Fig. 47). The cells of the middle tier divide further to form the endosperm tissue (Fig. 48).

During the later stages in the development of the seed the two cells of the chalazal haustorium are vacuolated and become pointed towards the lower end (Fig. 49). They penetrate the nucellar tissue which becomes disorganised and appear as dark irregular masses.

The micropylar haustorium is first initiated by the two large topmost cells of the endosperm, which later become prominent and extend into the micropyle (Figs. 50, 51). At the same time the remaining endosperm cells become elongated and arranged in a biseriate row with dense contents and conspicuous nuclei. These cells connect the micropylar haustorium with the remaining endosperm tissue.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Figs. 67–103

FIGS. 55–113. *Tecoma stans.*—Figs. 55–61. Longitudinal sections of progressively older flower buds × 30. Fig. 62–66. Transverse sections of the inflorescence axis to show the origin of the flowers × 24. Figs. 67–88. Cross sections of a flower from below upwards showing arrangement of floral parts × 24. Fig. 89. Two-celled archesporium × 400. Fig. 90. Megaspore mother cell in late telophase × 679. Fig. 91. Dyad × 679. Fig. 92. Dyad cells dividing × 679. Fig. 93. Linear tetrad × 679. Fig. 94. Two-nucleate embryo sac × 679. Fig. 95. Four-nucleate embryo sac × 679. Fig. 96. Mature embryo sac; note the integumentary tapetum × 679. Fig. 97. A stage in double fertilisation; note the conspicuous cells at the chalazal end of the embryo sac × 485. Fig. 98. Primary endosperm nucleus in division × 485. Figs. 99–107. Stages in the development of the endosperm × 485. Fig. 108. Chalazal haustorium composed of four large cells; note the nutritive tissue at the chalazal region × 679. Fig. 109. Diagram showing the nutritive tissue, conducting strand and the four regions of the endosperm; note the development of the wings at the micropylar and chalazal ends respectively × 70. Fig. 110. Chalazal wing × 301. Fig. 111. Micropylar wing × 485. Fig. 112. Winged seed × nat. size. Fig. 113. A portion of the wing enlarged × 485.

In *Tecoma*, after the formation of the four-celled endosperm, the two cells of the chalazal tier divide transversely (Fig. 103) resulting in an endosperm of six cells arranged in three tiers of two cells each (Fig. 104). Immediately, the two basal cells undergo one more vertical division at right angles to the previous division, thus resulting in a chalazal haustorium of four cells (Fig. 105). The two cells of the middle tier divide further to form the endosperm proper (Figs. 106, 107).

The chalazal haustorium is made of four aggressive cells with their tips pointed towards the chalazal end (Fig. 108). They are densely filled with cytoplasmic contents and show characteristic vacuolisation. As in *Paramentiera* they also penetrate into the chalaza.

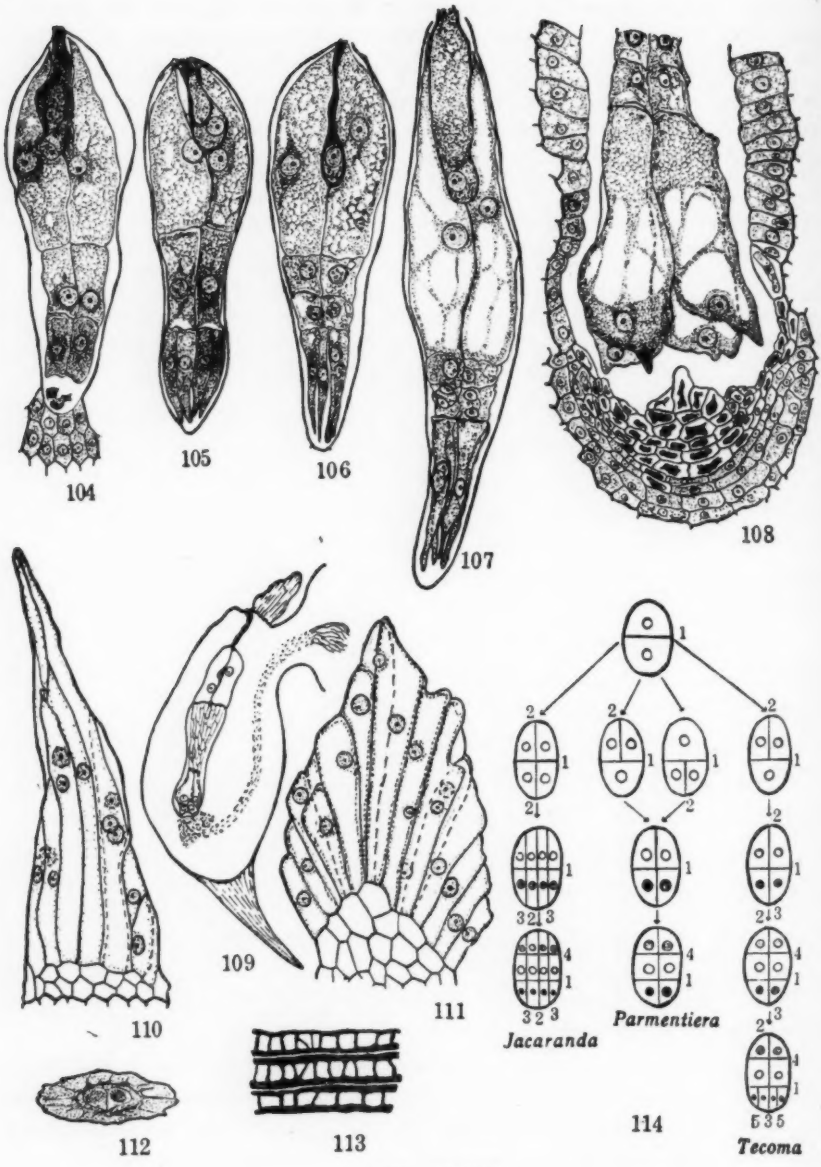
The two micropylar cells of the endosperm are non-aggressive (Fig. 107) as in *Jacaranda* and *Catalpa bignonioides* (Mauritzon, 1935).

The sequence of divisions of endosperm development in the three genera studied by me is diagramatically represented in Fig. 114.

## STRUCTURE OF THE WING OF THE SEED

The fruit is a woody follicle containing a large number of winged seeds (Figs. 16, 53, 112).

In *Jacaranda* the seed is trigonous with an equatorial fringe forming the membranous wing. In the initial stages the wing is composed of merely a group of elongated epidermal cells at the chalazal and micropylar ends (Fig. 21). As development proceeds, the elongation extends all round, resulting in the formation of a one-layered membranous wing showing a prominently ribbed structure.




FIGS. 104–114

FIG. 114. Chart showing the origin of the endosperm haustoria in some members of Bignoniaceæ.

Microscopic studies of the development of the wing indicate that it is formed by the vertical elongation of the outer epidermal cells of the testa. These are at first rectangular as seen in surface view but later become long and cylindrical. Due to a lateral coalescence of these elongated cells, a membranous wing is formed. At an early stage the cells of the wing have granular cytoplasm and conspicuous nuclei (Fig. 17). Later the cytoplasm becomes limited to the periphery and the nucleus stains less densely (Fig. 18). At the same time thickenings are laid along the wall (Fig. 18), interrupted by simple pits (Fig. 19). Through these pits adjacent cells are connected by delicate, simple or branched cytoplasmic strands (Fig. 19). Some of the cells show a brown tinge. In such cells, in addition to oil globules, crystals with radiating rays are also present (Fig. 20) and the cytoplasm appears brown. It is these cells which give the ribbed appearance to the wing.

In *Parmentiera* the seed is small and the wing is formed by the close juxtaposition of palisade layers of cylindrical to cuboid cells (Fig. 54).

In *Tecoma*, the seed is ovate-ellipsoidal, with a membranous wing which is comparable to that of the two general described above as regards the mode of its development and structure. In early stages, the cells are uninucleate with scanty cytoplasm (Figs. 110, 111). In later stages the radial walls of these elongated epidermal cells get conspicuously thickened by the deposition of lignin. Consequent to the lignification of the cell walls, the nucleus degenerates. Thickenings appearing like cross partitions or diaphragms are seen in mature stages (Fig. 113).

## Discussion

Engler and Prantl (1897) include Bignoniaceæ under the Tubifloræ while Bentham and Hooker (1862–67) and Hutchinson (1926) place it under Personales in their systems of classification. Thus in both the systems of classification, the place accorded to the Bignoniaceæ is somewhat similar. Wernham (1913) in his treatise on the floral evolution among the Sympetalæ, traces the origin of the Bignoniaceæ from the apocynal plexus and places it among the Tubifloaræ. The occurrence of the dendroid habit and woody follicles in several members of the Bignoniaceæ are reminescent of similar conditions present in the Apocynaceæ. However, when embroyological data are taken into consideration, the Bignoniaceæ show considerable differences and seem to be far removed from the Apocynaceæ. On the other hand, Bignoniaceæ show closer resemblances with the families such as the Verbenaceæ and Boraginaceæ included under Engler's Tubifloræ.

The following facts may be considered in supporting this close relationship between the Bignoniaceæ, Boraginaceæ and Verbenaceæ. A cellular

endosperm is common to all the families included under Tubifloræ. The endosperm of the Apocynaceæ on the other hand, is nuclear and becomes cellular only in later stages.

As has already been stated, the most noteworthy feature in the embryology of the Bignoniaceæ is the endosperm development. The development of endosperm in the plants under study follows the *Catalpa* type of Mauritzon (1935) with certain minor variations. Mauritzon, who studied only the early stages of development in *Jacaranda*, states that it resembles that of *Catalpa bignonioides* and presumes that in later stages it may resemble that of *C. Kæmpferi*. The present investigation confirms Mauritzon's assumption that the endosperm development in *Jacaranda* conforms to the *Catalpa* type, but it shows a greater resemblance wth *C. bignonioides* than with *C. Kæmpferi* regarding the details of development of the micropylar haustoria.

*Parmentiera* possesses a two-celled and aggressive chalazal haustorium as in *Bignonia megapotamica* (Swamy, 1941). However, the micropylar haustorium of *Parmentiera* consists of two fairly prominent and elongated cells while that of *Bignonia* comprises a single four-nucleate cell.

*Tecoma* resembles *Catalpa* in having a four-celled chalazal haustorium but differs in having only two cells at the micropylar end.

In addition to the integumentary tapetum and the endosperm haustoria which play an important role in the nutritional mechanism of the seed, another important structure is the nutritive tissue organised at the chalazal region of the ovule. During later stages of the development of the embryo-sac, these cells show conspicuous nuclei and dense contents, and become very prominent. A similar chalazal zone of radially arranged cells has been reported by Swamy (1941) in *Bignonia*, and Krishna Iyengar (1947) and Kausik (1940) in the Scrophulariaceæ and Hydrocharitaceæ respectively. These cells probably serve to conduct food material to the developing embryo.

As reported by Swamy (1941) for *Bignonia*, a strand of cells with dense protoplasmic contents is met with in all the forms under study (Figs. 21, 52, 109). This strand connects the chalazal group of cells with the vascular traces at the base of the ovule.

## Summary

An account of the embryology of *Jacaranda mimosæfolia*, *Kigelia pinnata*, *Parmentiera cerifera* and *Tecoma stans* is presented in this paper.

The organogeny has been studied in detail in *Tecoma*. The floral parts arise in acropetal succession. In each whorl the anterior parts precede the posterior in development.

The ovary is superior, bilocular with numerous ovules attached to an axile placenta. Sometimes hemitropous ovules are also met with, as in *Kigelia* and *Parmentiera*. The ovules have a single massive integument, the innermost layer of which forms the integumentary tapetum. Usually the tapetum lies only in the non-dilated chalazal portion of the embryo sac. A nutritive tissue consisting of radiating cells at the chalaza is present in the forms studied.

The single hypodermal archesporial cell functions directly as the megaspore mother cell. A linear tetrad of megaspores is formed, but occasionally a T-shaped, an isobilateral or even a decussate arrangement of megaspores is seen.

The development of the embryo sac conforms to the *Polygonum* type. The synergids are hooked and show pointed apices with their nuclei situated in the centre. The antipodals are organised as definite cells. Except in *Kigelia*, they persist during early stages of endosperm development. The two polar nuclei unite before fertilisation in the upper portion of the embryo sac to form the secondary nucleus. Double fertilisation has been observed in *Tecoma*.

The endosperm is *ab initio* cellular and follows the *Catalpa* type of Mauritzon (1935) with minor variations. The chalazal haustorium is usually aggressive. It is four-celled in *Jacaranda* and *Tecoma* and two-celled in *Parmentiera*. The upper end of the endosperm is usually non-aggressive. It is four-celled in *Jacaranda* and two-celled in *Parmentiera* and *Tecoma*. In *Parmentiera*, however, the uppermost cells of the endosperm become elongated in later stages, serving probably as weak haustoria. The remaining cells succeeding the haustorium become elongated and connect the micropylar haustorium with the remaining endosperm tissue.

The development and structure of the wing have been studied in the seeds of *Jacaranda*, *Parmentiera* and *Tecoma*.

In conclusion, I wish to express my gratefulness to Dr. L. N. Rao, Professor of Botany, Central College, Bangalore, for guidance and facilities. I am indebted to Dr. P. Maheshwari for going through the manuscript and to Dr. M. J. Thirumalachar and Sri. K. Subramanyam for helpful suggestions.

## Literature Cited

Bentham, G. and Hocker, J. D. .. *Genera Plantarum*, London, 1862–67.

Engler, A. and Prantl, K. .. *Die natürlichen pflanzenfamilien*, Jena, 1897.

Hutchinson, J. .. *Families of flowering Plants, I*, London, 1926.

Kausik, S. B. .. "A contribution to the embryology of *Enalus acoroides* (L. fil.) Steud," *Proc. Indian Acad. Sci.*, B, **11**, 1940, 83-99.

Krishna Iyengar, C. V. .. "Development of seed and its nutritional mechanism in the Scrophulariaceæ," *J. Mysore Univ.*, 1947, **7**, 83-98.

Maheshwari, P. .. "A critical review of the types of embryo sacs in Angiosperms," *New Phytol.*, 1937, **36**, 359–417.

——— .. "Recent work of the types of embryo sacs in Angiosperms," *J. Indian Bot. Soc.*, 1941, **20**, 229–61.

——— .. "The Angiosperm embryo sac," *Bot. Rev.*, 1948, **14**, 1-56.

Mauritzon, J. .. "Etwas über die Embryologie der Bignoniaceen," *Bot. Not.*, 1935.

Swamy, B. G. L. .. "Contribution to the life history of *Bignonia megapatomica*," *J. Indian Bot. Soc.*, 1941, **20**, 399–405.

Venkatasubban, K. R. .. "Cytological studies in Bignoniaceæ," *Diss.* Annamalai University, 1944.

——— .. "Cytological studies in Bignoniaceæ. IV. The cytology of *Dolichondrone Rheedii* Seem., and allied genera," *Proc. Indian Acad. Sci.*, 1945, B, **21**, 77–92.

Wernham, H. F. .. "Floral evolution with particular reference to the sympetalous dicotyledons," *New Phytol.*, 1913.

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# STUDIES IN CROP PHYSIOLOGY

## On Some Physiological Attributes of Transpiring Surface in Relation to Drought Resistance in Sugarcane

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### Introduction

Growth performance of crop plants under adverse climatic conditions is largely determined by the water relation of the plants and the physioloy of leaf growth exhibited under these conditions. Evidences collected in these directions show that the extent of transpiring surface influences, fruit size,[11, 14, 22, 26] fruit production,[24] fruit bud differentiation,[30] and dry matter residue.[4] Efficiency of leaf to increase dry matter accumulation during vegetative phase, however, remains constant for majority of plants with the result that despite structural variations, $CO_2$ utilized per unit area remains the same under all conditions.[13] Further, internal factors controlling net assimilation rate have been suggested to be independent of age during period of vegetative activity,[9, 12] though evidences on the contrary, are also on record.[3, 39, 40, 41]

Leaf growth depends considerably upon weather conditions in general,[1, 32] though individual blades on the same plant might occasionally exhibit most diverse kinds of growth without any discernible rule.[23] Even in a single day, variations to the extent of one per cent. in area have been recorded; no relation between differences in area and atmospheric humidity have been noticed.[6] Temperature is also known to affect rate of production of leaf initials,[2, 10, 37] but subsequent cell division and extension appear less related. In Lemna, frond area is independent of temperature. Longitudinal growth of paddy leaves is more related to soil temperature than air temperature.[28] Light, on the contrary, seems to have a photochemical action on some metabolite in leaves which gets converted into materials essential for growth and cell division.[8, 9, 10, 25, 38] Its effect upon cell size and total number of cells of mature leaves appears considerable.[34, 35, 36] In low intensities, it affects formation of leaf initials and multiplication rate in Lemna.[2, 7] Leaf area is affected by manurial treatments differently in different species.[21] Auxins appear more effective than heteroauxin.[5]

Age and development of plants affect physiology of leaf growth. In sugarcane, relation between dry matter output and leaf area is negative during pre-monsoon period while during the monsoon it is highly positive.[16] Close correlation between area of seed leaf, width of seed leaf, breadth of widest foliage leaf and total leaf area on plants is recorded in hardy varieties of winter wheat.[17] Size of leaves is also related to drought resistance.[33] Sorghum is suggested to be more drought resistant than corn mainly because it is less leafy.[18] In deciduous plants too, species with narrow leaves of low water content are recorded in resistant varieties.[19] Drought resistance is characterised by the capacity of species to withstand low ratio of water content to dry weight and very high saturation deficit,[27] and is known to change with environment and plant development.[31] Positive correlation between the period of appearance of tillers and drought resistance has been recorded.[20]

It therefore appears that development of transpiring surface is an inherent characteristic of the species depending largely upon the conditions of the environment. How far the physiological attributes of the individual leaves change with the development of leaf and to what extent such variations are related to the internal factors of tiller development on the one hand, and atmospheric factors on the other is little understood. It is, therefore, attempted to elucidate this relationship in certain drought resistant, medium drought resistant and drought susceptible forms of sugarcane. Physiological attributes so characteristic of drought resistant forms have been carefully determined on the basis of a series of measurements taken on the pre-monsoon, monsoon and post-monsoon tillers of different age and leaves of varying chronological order. The role of external factors in determining leaf development is also elucidated. Relevant data recorded in these directions are herein presented.

## Experimental Procedure

For purposes of present investigation, certain drought resistant, medium drought resistant and standard Coimbatore varieties were grown under known conditions of soil nutrition on the College Experimental Farm. These included Co 453, Co 205, Co 385, Co 421, Co 312, Co 313, Co 356, Co 213, Co 299, Co 290, CoS 76 and Rheora. Observations on leaf characteristics of these varieties were undertaken to elucidate (i) physiological characteristics of leaf during its expansion, (ii) characteristic of the fully expanded leaf on tillers of varying age, (iii) varietal differences of transpiring surface in relation to age of leaf and tiller development and lastly, (iv) effect of prevailing atmospheric conditions on the physiological attributes of fully expanded leaves from the pre-monsoon, monsoon and post-monsoon tillers.

Three bushes belonging to each of the above varieties were tagged towards the end of monsoon season for experimentation. Of these all shoots belonging to one of these bushes were divided in December 1946 into four classes: (i) mother shoot nine months old; (ii) tillers of pre-monsoon origin 6–8 months old; (iii) tillers of monsoon origin 3–6 months old; and (iv) tillers of post-monsoon origin 1–3 months old. In each of these classes at least 2–3 tillers were considered for comparative study of the effect of tiller age. For statistical analysis, however, only one representative type belonging to each class was selected at random from the sample already taken and classified as above. In the matter of classification of shoots into one of the above groups certain tests based on (i) height, (ii) girth of maximum size internode, (iii) leaf number and size and (iv) number of internodes exposed were employed. In view of marked differences in these directions it was relatively easy to demarcate one class of tillers from the other with fair degree of accuracy. Within each group, however, relative age was judged by height of the shoot. On each of these shoots the following characters were recorded: (i) total number of green leaves, (ii) leaf area of individual foliage, (iii) length and width of successive leaves, (iv) fresh and dry weight of leaves and (v) percentage water content on fresh weight basis. Total transpiring area was determined by planimeter. Dry weight was estimated by drying the samples to constant weight in an electric oven at 105° C. Measurement of leaf size was done after the manner indicated in a previous publication from this laboratory.[29]

Records on above physiological attributes of leaf were subjected to statistical analysis to assess the effect of different internal factors determining leaf development such as (i) inherent varietal characteristics, (ii) leaf age and (iii) tiller age. The investigations, in the first instance, involved a factorial study of the effect of 12 varieties, five leaf ages and four tiller ages, in all making up a total number of $12 \times 5 \times 4 = 240$ combinations of factor. Main effects and first order interactions due to these factors were estimated by the analysis of variance method as indicated in text tables. Summarised results of statistical analysis are presented in the text.

In the second part of the investigation, physiological attributes of maximum size leaf on all tillers of different varieties were taken for comparative study. In each variety, maximum size leaf on different tillers was picked up and physiological characteristics enumerated above were recorded to evaluate the effect of tiller age. How far a tiller which was initiated to development during the pre-monsoon period differed from a tiller of the monsoon or the post-monsoon stage, has been indicated in all the varieties. Leaf characteristics common to all varieties during the three critical periods

of growth, *viz.*, pre-monsoon, monsoon and post-monsoon have been compared with the prevailing atmospheric conditions in these seasons. The influence of climate on this important aspect of sugarcane physiology has been evaluated.

## Experimental Results

### *A. Effect of Leaf and Tiller Development on Leaf Length in Different Varieties*

Data recorded on the length of leaves have shown that main effects due to varieties, tillers and leaf age were highly significant. Among the first order interactions V × T, only showed significant effects (Table I *a*)

Table I. *Length of leaves (cm.) in relation to varieties, tiller age and leaf age*

(*a*) *Analysis of Variance*

| Due to | D. F. | S. S. | M. S. S. |
|---|---|---|---|
| Varieties .. | 11 | 59400·24 | 5400·02** |
| Tillers .. | 3 | 23361·60 | 7787·20** |
| Leaves .. | 4 | 50655·11 | 12663·77** |
| V × T .. | 33 | 17886·10 | 542·00** |
| V × L .. | 44 | 4201·89 | 95·49 |
| T × L .. | 12 | 2983·19 | 248·59 |
| Error .. | 132 | 14716·61 | 111·48 |
| Total .. | 239 | 173204·74 | |
| S.E./observation ; ±10·53 | | | |

(*b*) *Variety × Leaf age interaction*

| Varieties | Leaves | | | | | Mean (20 values) | Increases |
|---|---|---|---|---|---|---|---|
| | $L_1$ | $L_2$ | $L_3$ | $L_4$ | $L_5$ | | |
| Co 290 .. | 59·7 | 76·7 | 84·7 | 93·2 | 94·7 | 81·8 | .. |
| ,, 299 .. | 73·0 | 89·2 | 99·7 | 102·5 | 102·5 | 93·4 | −11·6 |
| ,, 453 .. | 78·0 | 95·0 | 111·0 | 129·0 | 124·2 | 107·4 | −14·0 |
| ,, 421 .. | 45·7 | 55·7 | 71·0 | 83·5 | 101·2 | 71·4 | 36·0 |
| ,, 312 .. | 81·0 | 108·5 | 116·5 | 127·2 | 127·2 | 112·1 | −40·7 |
| ,, 205 .. | 55·2 | 70·2 | 83·0 | 92·7 | 101·2 | 80·5 | 31·6 |
| ,, 313 .. | 49·0 | 64·0 | 71·0 | 68·0 | 80·0 | 66·4 | 14·1 |
| ,, 356 .. | 66·0 | 79·7 | 89·0 | 96·7 | 114·7 | 89·2 | −22·8 |
| ,, 385 .. | 82·5 | 95·7 | 105·2 | 114·0 | 127·0 | 104·9 | −15·7 |
| ,, 213 .. | 64·7 | 80·5 | 90·7 | 98·7 | 100·0 | 86·9 | 18·0 |
| Cos 76 .. | 50·0 | 59·7 | 67·5 | 76·5 | 89·7 | 68·7 | 18·2 |
| Rheora .. | 43·7 | 56·2 | 65·0 | 74·0 | 83·2 | 64·4 | 4·3 |
| Mean of 48 values | 62·3 | 77·8 | 87·8 | 96·3 | 103·8 | C.D. at 5%: | var. : ± 6·44 |
| Increases .. | −15·5 | −10·0 | −8·5 | −7·5 | | | L. age : ± 4·20 |

*(c) Tiller age × Leaf age interaction*

| Tillers | Leaves | | | | | Mean (60 values) | Increases |
|---|---|---|---|---|---|---|---|
| | $L_1$ | $L_2$ | $L_3$ | $L_4$ | $L_5$ | | |
| $T_1$ .. | 64·0 | 80·5 | 93·6 | 104·4 | 106·0 | 89·7 | .. |
| $T_2$ .. | 64·6 | 83·1 | 95·2 | 105·1 | 120·8 | 93·8 | −4·1 |
| $T_3$ .. | 68·6 | 85·0 | 93·6 | 98·0 | 105·3 | 90·1 | 3·7 |
| $T_4$ .. | 52·2 | 61·7 | 68·9 | 77·7 | 83·0 | 68·7 | 21·4 |
| Mean of 48 values | 62·3 | 77·8 | 87·8 | 96·3 | 103·8 | C.D. at 5% : T. age : ± 3·64 | |
| Increases .. | −15·5 | −10·0 | −8·5 | −7·5 | | L. age : ± 4·20 | |

$T_1$ : Mother shoot ; $T_3$ : Monsoon tiller
$T_2$ : Pre-monsoon tiller ; $T_4$ : Post-monsoon tiller

*(d) Variety × Tiller age interaction*

| Varieties | Tillers | | | | Mean (20 values) | Increases |
|---|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | | |
| Co 290 .. | 88·6 | 83·2 | 91·6 | 64·0 | 81·8 | .. |
| „ 299 .. | 106·6 | 95·0 | 95·6 | 76·4 | 93·4 | −11·6 |
| „ 453 .. | 125·6 | 118·0 | 110·4 | 75·8 | 107·4 | −14·0 |
| „ 421 .. | 76·0 | 77·4 | 76·2 | 56·2 | 71·4 | 36·0 |
| „ 312 .. | 100·4 | 140·0 | 118·6 | 89·4 | 112·1 | −40·7 |
| „ 205 .. | 86·6 | 72·6 | 75·4 | 87·4 | 80·5 | 31·6 |
| „ 313 .. | 73·4 | 76·0 | 63·6 | 52·6 | 66·4 | 14·1 |
| „ 356 .. | 87·4 | 105·2 | 92·0 | 72·4 | 89·2 | −22·8 |
| „ 385 .. | 112·2 | 106·4 | 129·8 | 71·2 | 104·9 | −15·7 |
| „ 213 .. | 79·6 | 106·0 | 88·0 | 74·2 | 86·9 | 18·0 |
| Cos 76 .. | 69·4 | 79·8 | 74·8 | 50·8 | 68·7 | 18·2 |
| Rheora .. | 71·2 | 66·2 | 65·8 | 54·6 | 64·4 | 4·3 |
| Mean (60 values) .. | 89·7 | 93·8 | 90·1 | 68·7 | C.D. at 5% : var. : ± 6·44 | |
| Increases .. | −4·1 | 3·7 | 21·4 | | T age : ± 3·64 | |

Increase in leaf development was associated in general, with greater length of leaf commencing from apical leaf; significant increases in length were recorded between successive pair of leaves in the chronological order down the stem up to the fourth leaf stage. Between the fourth and fifth leaves, leaf length did not differ significantly among the varieties, average leaf length was maximum in Co 312 and minimum in Rheora. Co 312, Co 453 and Co 385 showed significantly higher leaf length than others. Similarly Co 421 Cos 76, Co 313 and Rheora exhibited significantly lower length of the leaves; others were intermediate between these two limits. Difference between the three classes were found to be statistically significant; varieties

belonging to each of these groups did not always show significant differences (Table I *b*). Effect of tiller age was also found to be significant. Pre-monsoon tillers showed maximum leaf size which differed significantly from mother shoot or from tillers of monsoon and post-monsoon stages (Table I *c* and *d*).

### *B. Effect of Leaf and Tiller Development on Width of Leaf in Different Varieties*

On breadth of individual leaves, all the main effects due to varieties, tiller age and leaf age were highly significant (Table II *a*). Width of the leaf

TABLE II. *Width of leaves (cm.) in relation to varieties, tiller age and leaf age*

(*a*) *Analysis of Variance*

| Due to | D. F. | S. S. | M. S. S. |
|---|---|---|---|
| Varieties .. | 11 | 72·81 | 6·61** |
| Tillers .. | 3 | 31·42 | 10·47** |
| Leaves .. | 4 | 16·77 | 4·19** |
| V × T .. | 33 | 4·94 | 0·14* |
| V × L .. | 44 | 5·44 | 0·12 |
| T × L .. | 12 | 2·16 | 0·18 |
| Error .. | 132 | 10·46 | 0·079 |
| Total .. | 239 | 144·00 | |
| S.E./observation : | ± 0·026 | | |

(*b*) *Variety × Leaf age interaction*

| Varieties | Leaves | | | | | Mean (20 values) | Increases |
|---|---|---|---|---|---|---|---|
| | $L_1$ | $L_2$ | $L_3$ | $L_4$ | $L_5$ | | |
| Co 290 .. | 3·62 | 3·85 | 3·92 | 4·00 | 4·02 | 3·8 | .. |
| ,, 299 .. | 2·52 | 2·85 | 2·82 | 3·12 | 3·25 | 2·9 | 0·9 |
| ,, 453 .. | 2·50 | 3·02 | 4·07 | 4·32 | 4·05 | 3·5 | −0·6 |
| ,, 421 .. | 2·45 | 2·57 | 2·57 | 2·97 | 3·37 | 2·7 | 0·8 |
| ,, 312 .. | 1·92 | 2·15 | 2·35 | 2·42 | 2·42 | 2·2 | 0·5 |
| ,, 205 .. | 2·05 | 2·55 | 2·87 | 2·97 | 3·02 | 2·6 | −0·4 |
| ,, 313 .. | 1·80 | 2·47 | 2·60 | 2·82 | 2.82 | 2·5 | 0·1 |
| ,, 356 .. | 2·15 | 2·37 | 2·45 | 2·55 | 2·67 | 2·4 | 0·1 |
| ,, 385 .. | 3·50 | 3·65 | 3·70 | 3·70 | 3·65 | 2·6 | −0·2 |
| ,, 213 .. | 2·75 | 3·00 | 3·15 | 3·30 | 3·32 | 3·1 | −0·5 |
| Cos 76 .. | 2·00 | 2·22 | 2·42 | 2·50 | 2·65 | 2·3 | 0·8 |
| Rheora .. | 1·97 | 2·12 | 2·32 | 2·57 | 2·65 | 2·3 | 0·0 |
| Mean (48 values) | 2·4 | 2·7 | 2·9 | 3·1 | 3·1 | C.D. at 5% : var. : ± 0·014 | |
| Increases .. | −0·3 | −0·2 | −0·2 | 0·0 | | L. age : ± 0·0084 | |

(*c*) *Tiller age* × *Leaf age interaction*

| Tillers | Leaves | | | | | Mean (60 values) | Increases |
|---|---|---|---|---|---|---|---|
| | $L_1$ | $L_2$ | $L_3$ | $L_4$ | $L_5$ | | |
| $T_1$ .. | 2·5 | 3·0 | 3·3 | 3·5 | 3·5 | 3·2 | .. |
| $T_2$ .. | 2·5 | 2·9 | 3·3 | 3·4 | 3·4 | 3·1 | 0·1 |
| $T_3$ .. | 2·5 | 2·7 | 2·8 | 2·9 | 3·0 | 2·8 | 0·3 |
| $T_4$ .. | 2·0 | 2·2 | 2·2 | 2·4 | 2·5 | 2·3 | 0·5 |
| Mean (48 values) | 2·4 | 2·7 | 2·9 | 3·1 | 3·1 | C.D. at 5%: T. age: ± 0·0084 L. age: ± 0·0084 | |
| Increases .. | | −0·3 | −0·2 | −0·2 | 0·0 | | |

(*d*) *Variety* × *Tiller age interaction*

| Varieties | Tillers | | | | Mean (20 values) | Increases |
|---|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | | |
| Co 290 .. | 4·20 | 4·20 | 4·06 | 3·08 | 3·8 | .. |
| „ 299 .. | 3·22 | 3·18 | 3·16 | 2·10 | 2·9 | 0·9 |
| „ 453 .. | 4·26 | 3·92 | 3·40 | 2·80 | 3·5 | −0·6 |
| „ 421 .. | 3·36 | 2·92 | 2·88 | 2·00 | 2·7 | 0·8 |
| „ 312 .. | 2·48 | 2·54 | 2·20 | 1·80 | 2·2 | 0·5 |
| „ 205 .. | 3·10 | 2·66 | 2·52 | 2·50 | 2·6 | −0·4 |
| „ 313 .. | 2·88 | 2·92 | 2·28 | 1·94 | 2·5 | 0·1 |
| „ 356 .. | 2·80 | 2·92 | 2·14 | 1·90 | 2·4 | 0·1 |
| „ 385 .. | 3·94 | 3·78 | 3·80 | 3·04 | 2·6 | −0·2 |
| „ 213 .. | 3·40 | 3·72 | 2·76 | 2·54 | 3·1 | −0·5 |
| Cos 76 .. | 2·46 | 2·68 | 2·50 | 1·80 | 2·3 | 0·8 |
| Rheora .. | 2·50 | 2·38 | 2·32 | 2·12 | 2·3 | 0·0 |
| Mean (60 values) .. | 3·2 | 3·1 | 2·8 | 2·3 | C.D. at 5%: var.: ± 0·014 T. age: ± 0·0084 | |
| Increases .. | | 0·1 | 0·3 | 0·5 | | |

was significantly increased from first to second, second to third and third to fourth stages of leaf development. Fourth leaf in general, showed maximum breadth and was significantly superior to other leaves situated above. Differences between the fourth and fifth leaf stages were not significant at all (Table II *b*). Varieties showed distinct effects: Co 290, Co 453 and Co 213 exhibited maximum breadth and constituted a class significantly superior to others. Similarly Co 313, Co 356, Rheora, Cos 76 and Co 312 belonged to a group of low breadth leaves, significantly differing from varieties of Class I with maximum breadth or varieties of Class II with medium breadth. Effect of tiller age was again highly significant. Leaves on the mother shoot were broadest followed by leaves on pre-monsoon, monsoon

and post-monsoon tillers. Differences between each class were highly significant (Table II *c* and *d*).

*C. Effect of Leaf and Tiller Development on Leaf Area*[1] *in Different Varieties*

On total leaf surface all main effects, due to varieties, tiller age, and leaf age, and all first order interactions, V × T, V × L and T × L, were highly significant (Table III *a*). Tillers did not exhibit any significant effect on area

TABLE III. *Leaf area (sq. cm.) in relation to varieties, tiller age and leaf age*

(*a*) *Analysis of Variance*

| Due to | D. F. | S. S. | M. S. S. |
|---|---|---|---|
| Varieties .. | 11 | 3402007 | 309273·3** |
| Tillers .. | 3 | 1450830 | 483610·0** |
| Leaves .. | 4 | 2273610 | 568402·5** |
| V × T .. | 33 | 736857 | 22329·0* |
| V × L .. | 44 | 484047 | 11001·0** |
| T × L .. | 12 | 202587 | 16882·2** |
| Error .. | 132 | 437457 | 3314·06 |
| Total .. | 239 | 8987395 | |

S.E./observation: ±57·56

(*b*) *Variety × Tiller age interaction*

| Varieties | Tillers $T_1$ | $T_2$ | $T_3$ | $T_4$ | Mean (20 values) | Increases |
|---|---|---|---|---|---|---|
| Co 290 .. | 536·8 | 519·2 | 541·6 | 315·2 | 478·2 | .. |
| ,, 299 .. | 482·8 | 448·8 | 482·8 | 247·6 | 415·5 | 62·7 |
| ,, 453 .. | 836·8 | 668·0 | 619·6 | 354·0 | 619·6 | −204·1 |
| ,, 421 .. | 412·0 | 356·8 | 356·8 | 188·8 | 328·6 | 291·0 |
| ,, 312 .. | 346·4 | 470·8 | 348·8 | 277·2 | 360·8 | − 32·2 |
| ,, 205 .. | 330·4 | 272·8 | 254·4 | 282·4 | 285·9 | 75·8 |
| ,, 313 .. | 327·2 | 344·0 | 232·6 | 177·4 | 270·3 | 14·7 |
| ,, 356 .. | 346·0 | 423·6 | 248·8 | 189·2 | 301·9 | − 31·6 |
| ,, 385 .. | 620·8 | 574·0 | 692·8 | 357·6 | 561·3 | −259·4 |
| ,, 213 .. | 399·6 | 526·0 | 361·6 | 251·2 | 384·0 | 176·7 |
| Cos 76 .. | 270·4 | 393·2 | 295·2 | 134·4 | 273·3 | 111·3 |
| Rheora .. | 261·2 | 230·4 | 212·0 | 150·4 | 213·5 | 59·8 |
| Mean (60 values) .. | 430·8 | 435·6 | 434·8 | 243·7 | C.D. at 5%: var.: ± 35·36 | |
| Increases .. | −4·8 | 0·8 | 191·1 | | T. age: ± 20·72 | |

[1] By leaf area is meant the area exposed by both the leaf surface, which is equivalent to twice the surface area determined by planimeter.

(*c*) *Tiller age* × *Leaf age interaction*

| Tillers | Leaves | | | | | Mean (60 values) | Increases |
|---|---|---|---|---|---|---|---|
| | $L_1$ | $L_2$ | $L_3$ | $L_4$ | $L_5$ | | |
| $T_1$ .. | 229·3 | 354·1 | 462·1 | 534·8 | 573·8 | 430·8 | .. |
| $T_2$ .. | 243·6 | 352·6 | 449·3 | 532·1 | 600·3 | 435·6 | −4·8 |
| $T_3$ .. | 272·0 | 352·1 | 396·3 | 434·8 | 480·8 | 434·8 | 0·8 |
| $T_4$ .. | 148·5 | 206·1 | 236·0 | 297·6 | 333·5 | 243·7 | 191·1 |
| Mean (48 values) | 223·3 | 315·5 | 386·0 | 449·8 | 497·1 | C.D. at 5% : T. age : ± 20·72 L. age : ± 23·24 | |
| Increases .. | −92·2 | −70·5 | −63·8 | −47·3 | | | |

(*d*) *Variety* × *Leaf age interaction*

| Varieties | Leaves | | | | | Mean (20 values) | Increases |
|---|---|---|---|---|---|---|---|
| | $L_1$ | $L_2$ | $L_3$ | $L_4$ | $L_5$ | | |
| Co 290 .. | 286·5 | 414·5 | 500·5 | 588·5 | 604·0 | 478·2 | .. |
| ,, 299 .. | 297·5 | 375·5 | 398·0 | 481·0 | 525·5 | 415·5 | 62·7 |
| ,, 453 .. | 298·0 | 464·0 | 696·0 | 841·5 | 796·5 | 619·6 | −204·1 |
| ,, 421 .. | 174·5 | 210·0 | 276·0 | 392·5 | 590·0 | 328·6 | 291·0 |
| ,, 312 .. | 216·0 | 335·5 | 382·5 | 435·0 | 435·0 | 360·8 | − 32·2 |
| ,, 205 .. | 157·5 | 232·0 | 285·0 | 361·0 | 389·5 | 285·0 | 95·8 |
| ,, 313 .. | 130·7 | 223·0 | 294·7 | 322·0 | 381·0 | 270·3 | 14·7 |
| ,, 356 .. | 194·0 | 255·5 | 306·0 | 344·0 | 410·0 | 301·9 | − 31·6 |
| ,, 385 .. | 386·0 | 529·5 | 594·0 | 617·5 | 679·5 | 561·3 | −259·4 |
| ,, 213 .. | 257·5 | 354·0 | 410·5 | 443·0 | 458·0 | 384·6 | 176·7 |
| Cos 76 .. | 164·5 | 238·5 | 273·0 | 310·5 | 380·0 | 273·3 | 111·3 |
| Rheora .. | 118·0 | 155·5 | 215·5 | 262·0 | 316·5 | 213·5 | 59·8 |
| Mean (48 values) | 223·3 | 315·5 | 386·0 | 449·8 | 497·1 | C.D. at 5% : var. : ± 35·36 L. age : ± 23·24 | |
| Increases .. | −92·2 | −70·5 | −63·8 | −47·3 | | | |

of green leaves. No significant differences between leaf area on mother shoot, pre-monsoon tiller and monsoon tiller were recorded. Post-monsoon tiller, on the contrary, exhibited a significant reduction in total leaf surface. Varieties again could be classified into *Class I*: with high leaf area, *viz.*, Co 453, Co 385, Co 290 and Co 299; *Class II*: with medium leaf area, Co 213, Co 312 and Co 421 and lastly *Class III*: with lowest leaf area, *viz.*, Co 356, Co 205, Cos 76, Co 313 and Rheora (Table III *b*). With successive development of leaves, total leaf surface increased significantly even up to the fifth leaf stage when maximum transpiring surface was attained (Table III *c* and *d*). Differences between total surface of successive leaves were highly significant.

### *D. Effect of Leaf and Tiller Development on Dry Matter Accumulation in Different Varieties*

Dry weight of green foliage of plants was again significantly affected by all main effects due to varieties, tiller age, and leaf age (Table IV *a*). Mother tiller at an average exhibited maximum dry weight of individual leaf. Shoots of shorter age than this, exhibited lower dry weights. The younger the shoot the lower was the average dry weight of leaf. Varieties again showed significant differences in dry weight of leaves. Co 453 showed

TABLE IV. *Dry weight of leaves (gm.) in relation to varieties, tiller age and leaf age*

(*a*) *Analysis of Variance*

| Due to | D. F. | S. S. | M. S. S. |
|---|---|---|---|
| Varieties .. | 11 | 114·00 | 10·36** |
| Tillers .. | 3 | 69·97 | 23·32* |
| Leaves .. | 4 | 77·40 | 19·35** |
| V × T .. | 33 | 57·24 | 1·73 |
| V × L .. | 44 | 18·22 | 0·41 |
| T × L .. | 12 | 3·03 | 0·25 |
| Error .. | 132 | 136·70 | 1·03 |
| Total .. | 239 | 476·56 | |

S.E./observation : ±1·00

(*b*) *Variety × Tiller age interaction*

| Varieties | | Tillers | | | | Mean (20 values) | Increases |
|---|---|---|---|---|---|---|---|
| | | $T_1$ | $T_2$ | $T_3$ | $T_4$ | | |
| Co 290 .. | | 2·85 | 2·75 | 3·18 | 1·83 | 2·65 | .. |
| ,, 299 .. | | 2·14 | 1·63 | 2·23 | 1·09 | 1·77 | 0·88 |
| ,, 453 .. | | 5·75 | 4·36 | 2·91 | 1·18 | 3·55 | −1·78 |
| ,, 421 .. | | 3·02 | 2·60 | 2·48 | 0·87 | 2·24 | 1·31 |
| ,, 312 .. | | 2·04 | 2·44 | 1·93 | 1·27 | 1·92 | 0·32 |
| ,, 205 .. | | 1·83 | 1·41 | 1·28 | 1·47 | 1·50 | 0·42 |
| ,, 313 .. | | 1·38 | 1·86 | 1·07 | 0·72 | 1·25 | 0·25 |
| ,, 356 .. | | 2·43 | 2·03 | 1·19 | 0·84 | 1·62 | −0·37 |
| ,, 385 .. | | 3·03 | 2·66 | 3·53 | 1·44 | 2·67 | −1·05 |
| ,, 213 .. | | 2·06 | 2·72 | 1·60 | 1·15 | 1·88 | 0·79 |
| Cos 76 .. | | 1·38 | 1·85 | 1·55 | 0·56 | 1·34 | 0·54 |
| Rheora .. | | 1·39 | 1·15 | 0·95 | 0·64 | 1·03 | 0·31 |
| Mean (60 values) .. | | 2·44 | 2·29 | 1·99 | 1·09 | C.D. at 5%: var. : ± 0·616 | |
| Increases .. | | 0·15 | 0·30 | 0·90 | | T. age : ± 0·336 | |

(*c*) *Tiller age* × *Leaf age interaction*

| Tillers | Leaves | | | | | Mean (60 values) | Increases |
|---|---|---|---|---|---|---|---|
| | $L_1$ | $L_2$ | $L_3$ | $L_4$ | $L_5$ | | |
| $T_1$ .. | 1·12 | 1·99 | 2·73 | 3·13 | 3·24 | 2·44 | .. |
| $T_2$ .. | 1·19 | 1·78 | 2·43 | 2·90 | 3·14 | 2·29 | 0·15 |
| $T_3$ .. | 1·35 | 1·83 | 2·10 | 2·25 | 2·43 | 1·99 | 0·30 |
| $T_4$ .. | 0·63 | 0·87 | 1·01 | 1·40 | 1·53 | 1·09 | 0·90 |
| Mean (48 values) .. | 1·07 | 1·62 | 2·07 | 2·42 | 2·59 | C.D. at 5%: T. age: ± 0·336 L. age: ± 0·392 | |
| Increases .. | −0·55 | −0·45 | −0·35 | −0·17 | | | |

(*d*) *Variety* × *Leaf age interaction*

| Varieties | Leaves | | | | | Mean (20 values) | Increases |
|---|---|---|---|---|---|---|---|
| | $L_1$ | $L_2$ | $L_3$ | $L_4$ | $L_5$ | | |
| Co 290 .. | 1·51 | 2·13 | 2·64 | 3·46 | 3·53 | 2·65 | .. |
| ,, 299 .. | 1·22 | 1·68 | 1·90 | 2·02 | 2·05 | 1·77 | 0·88 |
| 453 .. | 1·36 | 2·44 | 4·05 | 5·01 | 4·89 | 3·55 | −1·78 |
| ,, 421 .. | 1·35 | 1·79 | 2·30 | 2·74 | 3·03 | 2·24 | 1·31 |
| ,, 312 .. | 1·10 | 1·84 | 2·13 | 2·28 | 2·28 | 1·92 | 0·32 |
| ,, 205 .. | 0·73 | 1·11 | 1·58 | 1·93 | 2·16 | 1·50 | 0·42 |
| ,, 313 .. | 0·53 | 1·09 | 1·29 | 1·51 | 1·86 | 1·25 | 0·25 |
| ,, 356 .. | 0·96 | 1·40 | 1·62 | 1·98 | 2·15 | 1·62 | −0·37 |
| ,, 385 .. | 1·70 | 2·42 | 2·78 | 2·97 | 3·47 | 2·67 | −1·05 |
| ,, 213 .. | 1·16 | 1·69 | 2·06 | 2·25 | 2·25 | 1·88 | 0·79 |
| Cos 76 .. | 0·80 | 1·13 | 1·37 | 1·55 | 1·82 | 1·34 | 0·54 |
| Rheora .. | 0·43 | 0·73 | 1·09 | 1·34 | 1·58 | 1·03 | 0·31 |
| Mean (48 values) .. | 1·07 | 1·62 | 2·07 | 2·42 | 2·59 | C.D. at 5%: var.: ± 0·616 L. age: ± 0·392 | |
| Increases .. | −0·55 | −0·45 | −0·35 | −0·17 | | | |

the highest dry weight; this was significantly higher than the dry weight of other varieties of Class II, *viz.*, Co 385, Co 290 and Co 421. Similarly varieties of Class II differed significantly from those of Class III, *viz.*, Co 312, Co 213, Co 299, Co 356, Co 205, Cos 76, Co 313 and Rheora. Class I exhibited significantly higher dry weight as compared to either Class II or Class III (Table IV *b*). Dry weight of leaves also increased with advance in leaf development reaching maximum weight in the fifth leaf from the top (Table IV *c* and *d*).

*E. Effect of Leaf and Tiller Development on Per cent. Water Content*

On the moisture content of leaves main effects of varieties alone showed significant effects. Interaction between V × T, and T × L were also signi-

ficant (Table V *a*). Increase in leaf age did not bring about any significant change in the moisture content though a general tendency of reduced water content with advance in age was noticeable (Table V *b*). Among th varieties Co 290 showed the maximum water content while Co 421 exhibited the least. The varieties could be classed as follows: (i) High water content, *e.g.*, Co 290, (ii) medium water content, *e.g.*, Co 356, Co 213, Co 299, Co 385, Co 312 and Co 453, and (iii) low water content, *e.g.*, Co 313, Cos 76, Co 205, Rheora and Co 421. Effect of tiller age was equally insignificant.

TABLE V. *Percentage moisture content on fresh weight basis in relation to varieties, tiller age and leaf age*

(*a*) *Analysis of Variance*

| Due to | D. F. | S. S. | M. S. S. |
|---|---|---|---|
| Varieties .. | 11 | 2795·38 | 254·12** |
| Tillers .. | 3 | 38·91 | 12·97 |
| Leaves .. | 4 | 41·58 | 10·39 |
| V × T .. | 33 | 1294·03 | 39·21** |
| V × L .. | 44 | 1083·48 | 24·62 |
| T × L .. | 12 | 3928·19 | 327·34** |
| Error .. | 132 | 2204·56 | 16·70 |
| Total .. | 239 | 11386·13 | |

S.E./observation : ±4·08

(*b*) *Variety × Leaf age interaction*

| Varieties | Leaves | | | | | Mean (20 values) | Increases |
|---|---|---|---|---|---|---|---|
| | $L_1$ | $L_2$ | $L_3$ | $L_4$ | $L_5$ | | |
| Co 290 .. | 78·50 | 74·70 | 73·17 | 67·47 | 65·07 | 71·78 | .. |
| ,, 299 .. | 64·62 | 64·55 | 59·67 | 62·67 | 62·92 | 62·89 | 8·89 |
| ,, 453 .. | 64·50 | 64·52 | 61·42 | 59·87 | 61·97 | 62·46 | 0·43 |
| ,, 421 .. | 54·40 | 53·37 | 57·42 | 59·77 | 61·30 | 57·25 | 5·21 |
| ,, 312 .. | 62·30 | 62·90 | 61·87 | 62·75 | 62·75 | 62·51 | −5·26 |
| ,, 205 .. | 60·02 | 59·65 | 60·50 | 60·17 | 59·70 | 60·01 | 2·50 |
| ,, 313 .. | 58·67 | 59·90 | 65·72 | 64·05 | 60·90 | 61·85 | −1·84 |
| ,, 356 .. | 66·47 | 64·90 | 65·82 | 62·17 | 62·27 | 64·33 | −2·48 |
| ,, 385 .. | 61·90 | 63·22 | 63·25 | 63·30 | 62·12 | 62·76 | 1·57 |
| ,, 213 .. | 64·92 | 62·92 | 62·87 | 63·42 | 62·97 | 63·42 | −0·66 |
| Cos 76 .. | 59·10 | 60·50 | 62·40 | 59·87 | 61·52 | 60·68 | 2·74 |
| Rheora .. | 59·17 | 62·42 | 56·60 | 57·40 | 59·75 | 59·07 | 1·61 |
| Mean (48 values) | 62·8 | 62·7 | 62·5 | 61·9 | 61·9 | C.D. at 5% : var. : ± 2·548 L. age : ± 1·624 | |
| Increases .. | 0·1 | 0·2 | 0·6 | 0·0 | | | |

(*c*) *Tiller age* × *Leaf age interaction*

| Tillers | Leaves | | | | | Mean (60 values) | Increases |
|---|---|---|---|---|---|---|---|
| | $L_1$ | $L_2$ | $L_3$ | $L_4$ | $L_5$ | | |
| $T_1$ .. | 62·9 | 63·6 | 62·9 | 61·3 | 60·7 | 62·34 | .. |
| $T_2$ .. | 64·1 | 61·6 | 62·2 | 62·6 | 62·8 | 62·70 | −0·36 |
| $T_3$ .. | 61·7 | 62·7 | 61·7 | 60·9 | 61·7 | 61·79 | 0·91 |
| $T_4$ .. | 62·7 | 63·1 | 63·2 | 62·7 | 62·3 | 62·83 | −1·04 |
| Mean (48 values) | 62·8 | 62·7 | 62·5 | 61·9 | 61·9 | C.D. at 5%: T. age : ± 1·456 | |
| Increases .. | 0·1 | 0·2 | 0·6 | 0·0 | | L. age : ± 1·624 | |

(*d*) *Variety* × *Tiller age intreaction*

| Varieties | Tillers | | | | Mean (20 values) | Increases |
|---|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | | |
| Co 290 .. | 73·82 | 77·24 | 67·44 | 68·64 | 71·78 | .. |
| ,, 299 .. | 62·64 | 64·18 | 61·12 | 63·62 | 62·89 | 8·89 |
| ,, 453 .. | 61·04 | 60·58 | 63·14 | 65·08 | 62·46 | 0·43 |
| ,, 421 .. | 60·66 | 53·64 | 53·80 | 60·92 | 57·25 | 5·21 |
| ,, 312 .. | 60·94 | 64·04 | 63·24 | 61·84 | 62·51 | −5·26 |
| ,, 205 .. | 59·84 | 61·00 | 58·78 | 60·42 | 60·01 | 2·50 |
| ,, 313 .. | 63·92 | 58·16 | 64·18 | 61·14 | 61·85 | −1·84 |
| ,, 356 .. | 60·04 | 64·54 | 64·36 | 68·38 | 64·33 | −2·48 |
| ,, 385 .. | 63·00 | 63·62 | 61·90 | 62·52 | 62·76 | 1·57 |
| ,, 213 .. | 62·70 | 64·80 | 67·22 | 58·98 | 63·42 | −0·66 |
| Cos 76 .. | 62·24 | 59·10 | 57·88 | 63·50 | 60·68 | 2·74 |
| Rheora .. | 57·24 | 61·56 | 58·50 | 58·98 | 59·07 | 1·61 |
| Mean (60 values) .. | 62·34 | 62·70 | 61·79 | 62·83 | C.D. at 5%: var. : ± 2·548 | |
| Increases .. | | 0·36 | 0·9 | 1·04 | T. age : ± 1·456 | |

Main shoot and tillers of pre-monsoon, monsoon, and post-monsoon origin, however, showed at an average moisture content of 61·79 to 62·83% (Table V *c* and *d*).

## Physiological Characteristics of Fully Expanded Maximum Size Leaves

In Co 421, pre-monsoon tillers invariably showed maximum length, breadth, area, fresh and dry weight and dry weight/area ratio. Post-monsoon tillers were poorer in these regards. Monsoon tillers occupied an intermediate position between these two. The greater the age, the higher appeared to be the values recorded for most of the characters (Fig. 1 *a*).

In Cos 76, post-monsoon tillers were again poorer in leaf size, area, fresh and dry weights, and dry weight/area ratio while the pre-monsoon tillers characteristically showed greater area, and fresh weight. In other

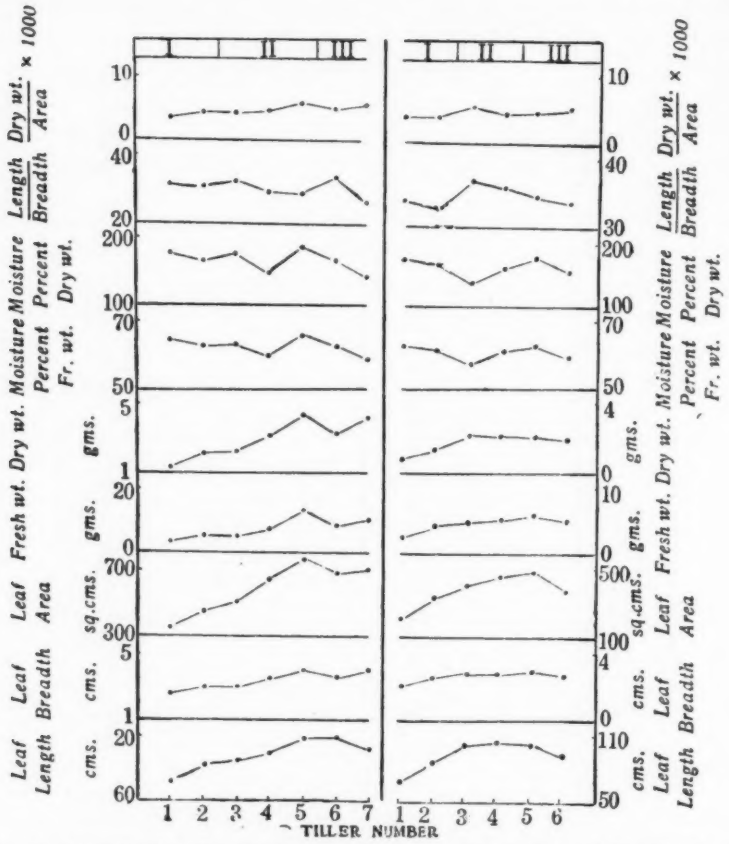


FIG. 1. Physiologcial Characteristics of Leaf of Co 421 (left *a*) and Cos 76 (right *b*) (Classes I, II and III represent tillers of post-monsoon, monsoon and pre-monsoon origin respectively)

respects monsoon and pre-monsoon tillers were more or less similar; L/B and dry weight/area ratios were maximum in case of fully expanded leaf on the third tiller belonging to the monsoon class (Fig. 1 *b*).

In Co 453, oldest tiller belonging to the pre-monsoon group, exhibited high dry weight/area and L/B ratios, greater dry weight, larger leaf area and maximum leaf size. Post-monsoon stand was poorest with respect to these

characters. Monsoon stand occupied an intermediate position as regards these. Water content on fresh weight and dry weight basis, however, was fairly high in monsoon stand and fairly low in pre-monsoon stand (Fig. 2 *a*).

In Co 385, pre-monsoon tiller invariably showed high values for all physiological attributes of leaf while post-monsoon was poorest in all these respects (Fig. 2 *b*). In Co 290, considerable differences were recorded with

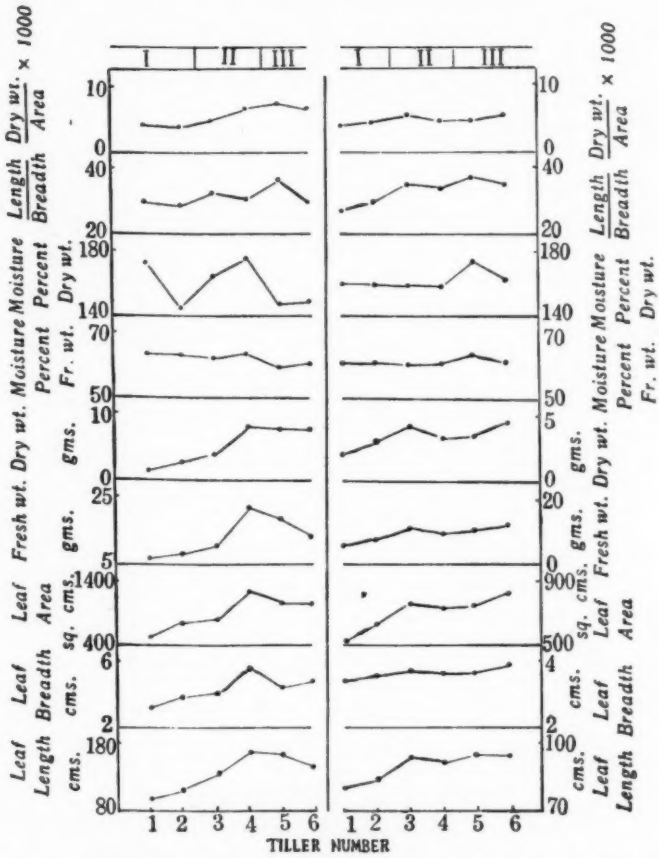


FIG. 2. Physiological Characters of Leaf of Co 453 (left *a*) and Co 385 (right *b*)

no systematic grouping. In some respects, *e.g.*, breadth, pre-monsoon stand was the best. In others, *e.g.*, dry weight, fresh weight, area, and water content, monsoon stand proved better. Post-monsoon stand behaved rather indifferently (Fig. 3 *a*).

In Co 312, distinctions were more obvious; size of leaves, area, fresh and dry weights were invariably high in the pre-monsoon stand while the post-monsoon shoots were the poorest. Tillers originated during monsoon period showed invariably higher water content (Fig. 3 *b*).

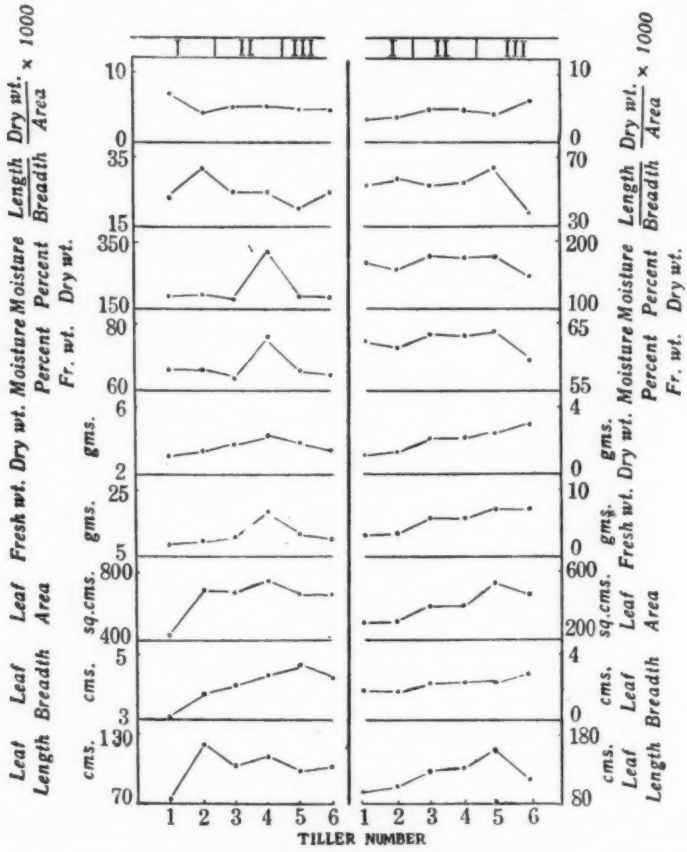


FIG. 3. Physiological Characters of Leaf of Co 290 (left *a*) and Co 312 (right *b*)

In Co 313, monsoon stand proved better in so far as length, breadth, area, fresh and dry weights and dry weight/area ratio were concerned. Pre-monsoon and post-monsoon stands were poorer in these regards. Water content in the post-monsoon stand was, however, higher than in leaves on older shoots of monsoon and pre-monsoon origin (Fig. 4 *a*).

In Co 356 again, pre-monsoon stand proved significantly better in so far as leaf size, area, fresh weight, and dry weight, were concerned. Tillers

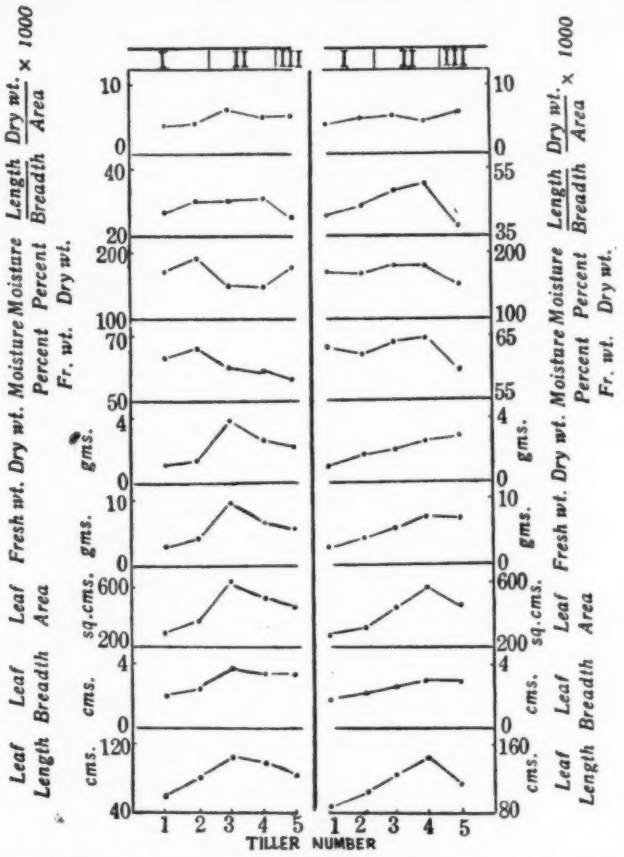


FIG. 4. Physiological Characters of Leaf of Co 313 (left *a*) and Co 356 (right *b*)

developed during pre-monsoon and monsoon period were invariably better than tillers developed during the post-monsoon period (Fig. 4 *b*).

In Rheora, results have been found to be more characteristic than those recorded for any of the varieties indicated above. All the characters of the leaves showed high values in the pre-monsoon stand, medium values in the monsoon stand, and low values in the post-monsoon stand. In other words, larger the tiller age the higher were the values recorded for different physiological characters (Fig. 5 *a*).

In Co 213, some of the characters like leaf size, area and fresh and dry weights were high in the pre-monsoon stand; others for instance, water content and L/B ratio were relatively higher in the monsoon stand. Post-monsoon shoots were poorest in all these attributes (Fig. 5 *b*).

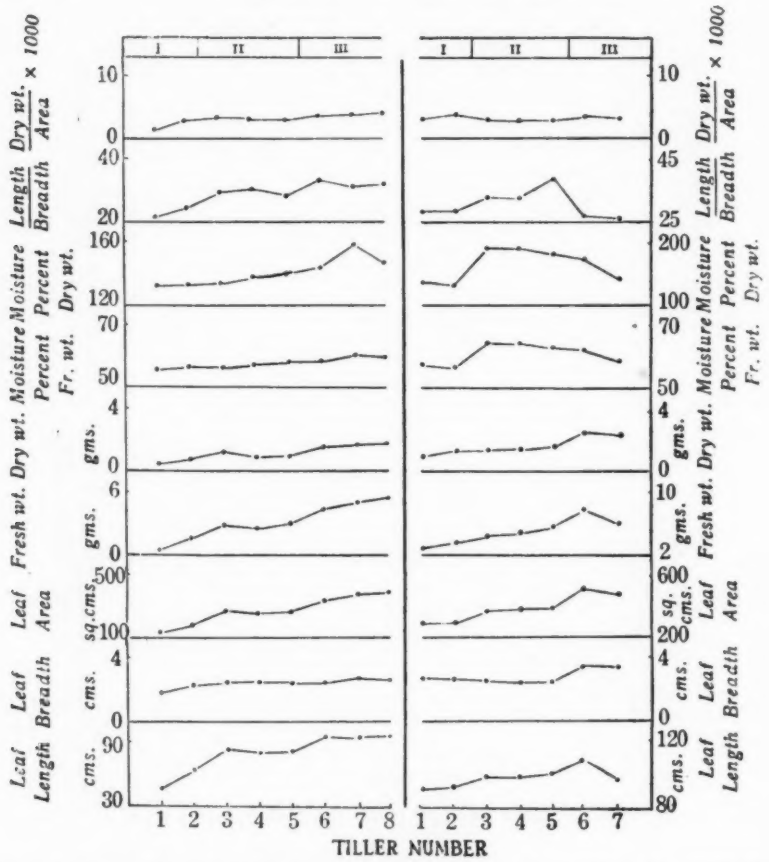


FIG. 5. Physiological Characters of Leaf of Rheora (left *a*) and Co 213 (right *b*)

In Co 205, responses were more or less identical to those noted in case of Rheora. In pre-monsoon shoots, values were high for different physiological characters. The monsoon stand occupied an intermediate position in majority of cases excepting water content where minimum values were recorded for tillers developed during the rainy season (Fig. 6 *a*).

In Co 299, pre-monsoon stand proved better in so far as leaf size, area, fresh and dry weights were concerned. Monsoon stand showed, on the other hand, higher water content, greater L/B ratio and relatively high dry weight/area ratio (Fig. 6 *b*).

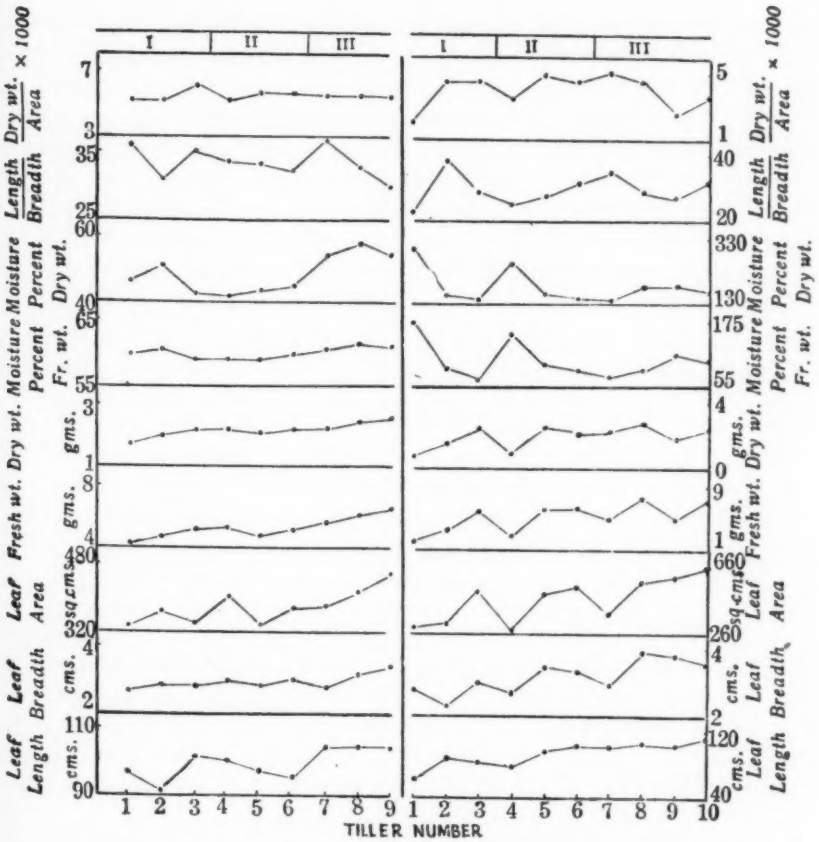


FIG. 6. Physiological Characters of Leaf of Co 205 (left *a*) and Co 299 (right *b*)

## DISCUSSION

Evidences collected on the effect of developmental factors on physiological characteristics of the leaf, have pointed out that specificity of the plant material has a significant role in determining most of the leaf characteristics such as length, breadth, area, dry weight and moisture content of the foliage. Tiller age and leaf age are equally significant except in case of

moisture content of the leaf where the effects were found to be insignificant. Interaction effects of V × L, V × T, and T × L were uniformly significant only in case of one attribute, *viz.*, the area of leaf surface. T × L showed significant results in case of moisture content alone. V × T affected besides area, the moisture content and width of the foliage significantly.

Analysis of individual effects of tillers on different leaf characteristics has also pointed out that the mother shoot or the pre-monsoon tiller, in general, showed highest average dry weight, area, length and breadth of individual green leaves. Post-monsoon shoots on the contrary, were poorest in these regards. Moisture content, however, did not differ significantly in relation to the period of initiation of the tiller. It, therefore, appears that in the post-monsoon tiller the conditions are less ideal for full development of the above leaf attributes whereas in the pre-monsoon tillers conditions are more congenial for better exposition of the developmental characteristics of the leaves.

Relative study of different leaf attributes has shown that varietal effects could be classified into three categories as follows:

*Class I.*—With high values of different leaf attributes, *viz.*, in Co 453 and Co 290.

*Class II.*—With medium values of different leaf attributes, *viz.*, in Co 299, Co 421, Co 385, Co 213, Co 312.

and *Class III.*—With low values of different leaf attributes, *viz.*, in Co 313, Co 356, Cos 76, Co 205 and Rheora.

A comparative study of these forms have shown that Rheora, a generally considered drought resistant variety of sugarcane, exhibits lower values of all the physiological attributes. If lower index of these characters is assumed to be associated with drought resistant behaviour of this variety, other sugarcane varieties such as Co 205, Co 313, Co 356 and Cos 76 also approach in their leaf characteristics a behaviour more or less similar to the behaviour of drought resistant Rheora.

Another important internal factor determining physiological behaviour of transpiring surface appears to be the stage of leaf development. Average values for different varieties have shown that increase in leaf age is usually associated with greater leaf size, larger area, higher dry weight and relatively lower moisture content of the foliage. The greater the stage of leaf development therefore, the higher are the values for such physiological attributes as leaf size, dry weight and area. While all these characters are affected significantly at least up to a certain stage of leaf development,

moisture content hardly indicates significant variations. These generalisations on developmental characters of leaves are true for most of the varieties under investigation. Transpiring surface thus undergoes changes in size, form and other characteristics with advance in age and is in consequence likely to bring about changes in physiological efficiency of leaf.

Further detailed studies on the effect of varying tiller age on the physiological characteristics of maximum size leaf have indicated that the season in which the shoot was brought into existence has a marked effect upon different physiological features. Post-monsoon tillers, as a general rule, exhibited lowest leaf size, smallest area and minimum weight of leaf. The pre-monsoon tillers on the other hand, in all the varieties under consideration, invariably exhibited higher physiological indices of leaf attributes studied in the present investigation. Monsoon stand, however, occupied more or less an intermediate position. It, therefore, appears that some kind of inhibitory effect on development of foliage is in operation during the post-monsoon period. What these inhibitory factors are, is yet little understood. Possibly, greater competition between the different shoots in the post-monsoon period for water and nutrient supply coupled with low light intensities and low temperature available inside the sugarcane fields may be responsible for poor development of foliage during this period. Well established shoots of the pre-monsoon and monsoon stand, it seems, divert most of the nutrients absorbed by roots towards development of foliage on these shoots. The large transpiring area on older shoots enables the sap to be diverted more and more to meet the requirements of leaves, leaving very little for translocation towards leaves borne on the post-monsoon tillers. Foliage on lately developed shoots is, therefore, handicapped in their development primarily due to these internal forces and secondly due to low intensities of illumination and temperature condition of environment. The less vigorous tillers succumb to these stresses while the relatively more vigorous ones try to exhibit a chequered development.

Compared with the conditions existing in the post-monsoon periods, the edaphic, climatic and internal factors are more favourable for foliage development during the pre-monsoon stage of the life cycle. There appears to be relatively little competition among the few shoots that develop during this period with the result that tillers of the pre-monsoon period develop more efficient conducting and transpiring surfaces. Their high efficiency of foliage growth is also related to relatively high nutrient status of the soil and more particularly to the high intensity of illumination, higher temperature and low humidity (Fig. 7)—all favourable conditions for the expansion of leaf surface. Stem tissues in pre-monsoon shoots are also likely

to be well developed both with respect to the mechanical and conducting tracts under these favourable conditions. Sound orientation of tissues, may at a later stage of the life-cycle, feed foliage with water and nutrients better on account of the high efficiency of the conducting tract. In the

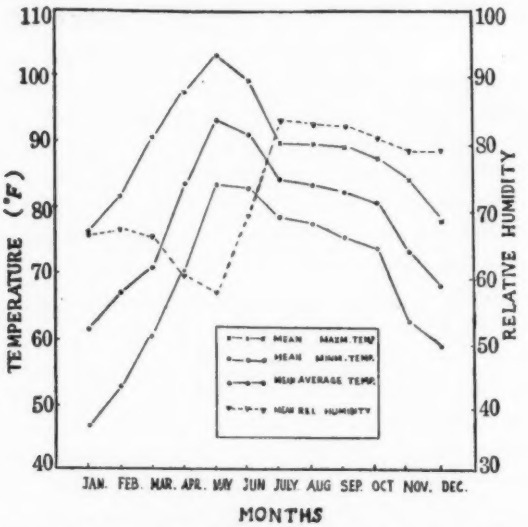


FIG. 7. Meteorological Conditions in different months of 1946 and recorded in the Meteorological Observatory of Benares Hindu University

pre-monsoon tillers there appears, therefore, to be a beneficial blending of internal tissue orientation with the result that growing leaves on these shoots do not suffer any handicap. Is it due to the high temperature and light conditions at the time of initiation of shoot primordia in pre-monsoon tillers ? If it be so, high temperature and light conditions at shoot formation are two primary requisites for better development of foliage at successive stages of plant's life cycle.

## SUMMARY

The paper deals with the effect of certain internal factors upon the development of leaf surface. The investigations were conducted on twelve varieties of sugarcane grown under field conditions. Measurement of various physiological attributes of the leaf were undertaken on shoots of pre-monsoon, monsoon and post-monsoon origin. Analysis of the effect of leaf age, tiller age, and environmental factors was conducted.

Statistical analysis of the data revealed that various physiological attributes of the leaf such as leaf length, leaf width, leaf area and dry matter

accumulation differed significantly with varieties, age of tillers and developmental stage of foliage.

Pre-monsoon shoots in general, produced leaves with highest dry weight, largest area and greater leaf size. Post-monsoon shoots were poorest. Conditions for full development of leaf surface were most favourable during pre-monsoon and least congenial in the post-monsoon period.

On the basis of the relative value for different physiological attributes varieties could be classified into three distinct classes:

*Class I.*—With high values of different leaf characteristics, *viz.*, Co 453 and Co 290.

*Class II.*—With medium values, *viz.*, Co 299, Co 421, Co 385, Co 213, and Co 312, and finally,

*Class III.*—With low values, *viz.*, Co 313, Co 356, Cos 76, Co 205, and Rheora.

If lower leaf indices in Rheora were to be taken as index of drought resistance, other sugarcane varieties of Class III, *viz.*, Co 313, Co 356, Cos 76 and Co 205 approached in their leaf characteristics a behaviour similar to the behaviour of drought resistant Rheora.

Among the factors determining physiological characteristics of transpiring surface, stage of leaf development and age of individual shoots are the most important. In general, the greater the leaf development upto a certain stage, the higher were the values for such attributes as leaf size, dry weight and leaf area. Similarly, the shorter the age of the tiller (post-monsoon shoots), the poorer was the development of leaf surface. This is accounted on the basis of (i) greater competition for water and nutrients and (ii) poorer light intensity and lower temperature during post-monsoon period.

Varietal characteristics of transpiring surface have been discussed with reference to the above factors and the major problem of drought resistance in sugarcane.

## Literature Cited

1. Aldrich, W. W., Crawford, C. L., Nixon, R. W. and Reuther, W. "Some factors affecting rate of date leaf elongation" *Proc. Amer. Soc. Hort. Sci.*, 1942, (1943) **41**, 77–84.
2. Ashby, E. and Oxley, T. A. .. "The interaction of factors in the growth of Lemna. IV. The analysis of the influence of light intensity and temperature on the assimilation rate and rate of frond multiplication," *Ann. Bot.*, 1935, **49**, 309-36.

3. Ballard, L. A. T. and Petrie, A. H. K. .. "Physiological ontogeny in plants and its relation to nutrition. I. The effect of nitrogen supply on the growth of the plant and its parts," *Austr. Jour. Exp. Biol. Medi. Sci.*, 1936, **14**, 135.

4. Chandler, W. H. .. "The dry matter residue of trees and their products in proportion to leaf area," *Proc. Amer. Soc. Hort. Sci.*, 1934, (1935) **31**, 39–56.

5. De Ropp, R. S. .. "Studies in physiology of leaf growth. I. The effect of various accessory growth factors on the growth of the first leaf of isolated stem tips of Rye," *Ann. Bot.* (N. S.), 1945, **9**, 369–81.

6. Goodall, D. W. .. "Diurnal changes in the area of Cacoa leaves," *ibid.*, 1947, **11**, 449–51.

7. Gorham, P. R. .. "Growth factor studies with *Sporodela polyrhize* (L.)" *Amer. Jour. Bot.*, 1945, **32**, 496–504.

8. Gregory, F. G. .. "Studies in the energy relations of plants. I. The increase in area of leaves and leaf surface of *Cucumis sativus*," *Ann. Bot.*, 1921. **35**, 94.

9. ——— .. "Effect of climatic conditions on the growth of barley," *ibid.*, 1926, **40**, 1–27.

10. ——— .. "Studies in the energy relations of plants. II. The effect of temperature on increase in area of leaf surface and dry weight of *Cucumis sativus*. I. The effect of temperature on the increase of leaf surface," *ibid.*, 1928, **42**, 469–507.

11. Gustafson, F. G. and Elnore, S. .. "Some relations between leaf area and fruit size in tomatoes," *Pl. Physiol.*, 1936, **11** (2), 445–51.

12. Heath, O. V. S. .. "Effect of age on net assimilation and relative growth rates in the cotton plant," *Ann. Bot.* (N.S.), 1937, **1**, 565–66.

13. ——— and Gregory, F. G. .. "The constancy of the mean net assimilation rate and its ecological importance," *ibid.*, 1938, **2**, 811–18.

14. Jones, I. D. .. "Further observation on influence of leaf area on fruit growth and quality in the peach," *Proc. Amer. Soc. Hort. Sci.*, 1932 (33), **29**, 34–38.

15. Kelly, V. W. .. "Relation of leaf form to transpiration rate and drought resistance in some deciduous fruits," *ibid.*, 1938 (39), **36**, 210–15.

16. Khanna, K. L. .. *Annual Report of the Sugarcane Research Scheme in Bihar*, Supt., Govt., Printing, Bihar, Patna, 1943.

17. Klages, K. H. W. .. "Metrical attributes and the physiology of hardy varieties of winter wheat," *Jour. Amer. Soc. Agron.*, 1926, **18** (7), 529–66.

18. Martin, J. H. .. "The comparative drought resistance of Sorghum and Corn," *ibid.*, 1930, **22** (12), 993–1003.

19. Mathur, R. N. .. "Analysis of certain varietal characteristics in sugarcane with reference to drought resistance and yield," *Proc. Ind. Sci. Cong.*, 1938, 215.

20. Mc Crory, S. A. .. "Heat and drought tolerance of sweet corn as influenced by tillers," *Proc. Amer. Soc. Hort. Sci.*, 1942, (1943), **40**, 424–28.

21. Morton, A. G. and Watson, D. J. "A physiological study of leaf growth," *Ann. Bot.* (N. S.), 1948, **12**, 281–310.

22. Murneek, A. E. .. "Relation of leaf area to fruit size and food reserves in apple seeds and branches," *Proc. Amer. Soc. Hort. Sci.*, 1932 (33), **29**, 230–34.

23. Papen, R. V. .. "Beitrage zur Kenntnis des Wachstums der Blattspreite," *Bot. Arch.* (Leipzig), 1935, **27** (2), 159–206.

24. Pickett, W. F. .. "Leaf area in relation to apple production," *Bienn. Rept. Kansas State Hort. Soc.*, 1934, **42**, 107–11.

25. Priestley, J. H. .. "The effect of brief light exposures upon etiolated plants," *New Phytol.*, 1925, **24**, 271–83.

26. Romsher, F. A. .. "Correlation between fresh weight and area of tomato leaves," *Proc. Amer. Soc. Hort. Sci.*, 1942, (1943), **40**, 482.

27. Runyon, E. H. .. "Ratio of water content to dry weight in leaves of Creosote bush," *Bot. Gaz.*, 1936, **97** (3), 518–33.

28. Sasaki, T. .. "On the relation of temperature to the longitudinal growth of leaves of rice plants," *Proc. Crop Sci. Soc. Japan*, 1927, **1**, 23–42.

29. Singh, B. N. .. "Age fertilizer effects upon certain aspects of the physiology and chemistry of sugarcane," *Proc. Ind. Acad. Sci.* **B**, 1941, **13** (5), 201–34.

30. Sproat, B. B., Darrow, G. M. and Beaumont, J. H. "Relation of leaf area to berry production in the strawberry," *Proc. Amer. Soc. Hort. Sci.*, 1935 (36), **33**, 389–92.

31. Stefanovskii, I. A. and Vetcheslova, E. M. "Prospects for breeding of spring wheat in dry regions of the south west," *Pl. Breeding and Seed Growing*, 1939, **9** (5), 10–12.

32. Swanson, A. F. .. "Relation of leaf area to grain yield in Sorghum," *Jour. Amer. Soc. Agron.*, 1941, **33** (10), 908–14.

33. Takasaki, T. .. "On relation between character and productivity proper to the varieties of potatoes," *Ann. Agric. Exp. Sta. Govt. Gen. Chosen.*, 1929, **4** (4), 209–20.

34. Watson, D. J. .. "The estimation of leaf area in field crops," *Jour. Agric. Sci.*, 1937, **27**, 474–83.

35. ———— .. "Comparative physiological studies on the growth of field crops. I. Variation in net assimilation rate and leaf area between species and varieties, and within and between years," *Ann. Bot.* (N. S.), 1947, **11**, 41–76.

36. ———— .. "Comparative physiological studies on the growth of field crops. II. The effect of varying nutrient supply on net assimilation rate and leaf area," *ibid.*, 1947, **11**, 375–407.

37. Watson, D. J. and Baptiste, E. C. D. .. "A comparative physiological study of sugarbeet and mangold with respect to growth and sugar accumulation. I. Growth analysis of the crop in the field," *ibid.*, 1938, **11** (2), 437–80.

38. Went, F. W. .. "Transplantation expts. with peas," *Amer. Jour. Bot.*, 1938, **25**, 44–61.

39. Williams, R. F. .. "Drift of net assimilation rate in plants," *Nature*, 1937, **140**, 1099–1100.

40. ———— .. "Physiological ontogeny in plants and its relation to nutrition. VI. Analysis of the unit leaf rate," *Aust. Jour. Exp. Biol. Medi. Sci.*, 1939, **17**, 123.

41. ———— .. "The physiology of plant growth with special reference to the concept of net assimilation rate," *Ann. Bot.* (N.S.), 1946, **10**, 41–72.

# ADDITIONS TO FUNGI OF MADRAS—IX

By T. S. Ramakrishnan, F.A.Sc. and K. Ramakrishnan
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Received September 27, 1949

(92) *Mycosphærella exaci* Ramakrishnan, T. S. and K., sp. nov.

Spots brown, amphigenous, irregular, isolated or confluent; perithecia numerous, amphigenous, subepidermal, ostiolate; asci hyaline, clavate, narrowed at the base, aparaphysate, 30 × 10 μ (18–39 × 7–12); ascospores 8, 1-septate, hyaline, cylindric to clavate, 13 × 3 μ (9–15 × 3).

Maculæ amphigenæ, irregulares, brunnei coloris, isolatæ vel confluentes; perithecia numerosa, amphigena, subepidermia, ostiolata; asci hyalini, clavati, basi attenuati, aparaphysati, 30 × 10 μ (18–39 × 7–12); ascosporidia 8, 1-septata, hyalina, cylindrica vel clavata, 13 × 3 μ (9–15 × 3).

On living leaves of *Exacum wightianum* Atn. (Gentianaceæ), Coonoor (Nilgiris), 20–6–1949, T. S. Ramakrishnan.

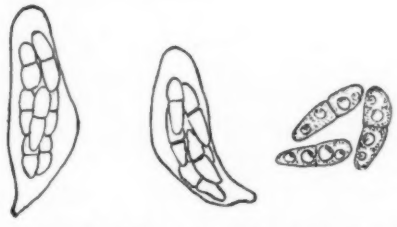

Fig. 1. *Mycosphærella exaci*—asci and ascospores ( × 350)

Many spots occur on a leaf, involving the whole leaf in some cases. Each spot has a central thin whitish circular portion surrounded by a brown water-soaked area. The white colour becomes prominent on drying. The perithecia occur in the central thin portion. The wall of the perithecium is made up of two or three layers of pale brown cells. The asci are narrowed at the base. The ascospores are lumped in the centre of the ascus. In each ascospore the upper cell is shorter than the lower. Prominent oil globules are present in each cell.

(93) *Mycosphærella gleicheniæ* Ramakrishnan, T. S. and K., sp. nov.

Spots amphigenous, grey or brown, irregular; perithecia amphigenous, isolated, innate, semi-erumpent, black, ostiolate; asci elliptic to obovate,

hyaline, aparaphysate, 26 × 12 μ (18–33 × 9–15); ascospores cylindric, hyaline, 1-septate, with obtuse ends 13 × 3 μ (12–15 × 3).

Maculæ amphigenæ, irregulares, cani vel, brunnei coloris; perithecia amphigena, isolata, innata, semierumpentia, nigra, ostiolata; asci elliptici vel obovati, hyalini, aparaphysati, 26 × 12 μ (18–33 × 9–15); ascosporidia cylindrica, hyalina, 1-septata, apice obtuso, 13 × 3 μ (12–15 × 3).

On living leaves of *Gleichenia linearis* Bedd. (Filicineæ), Coonoor (Nilgiris), 29–5–1948, T. S. Ramakrishnan and K. Ramakrishnan.

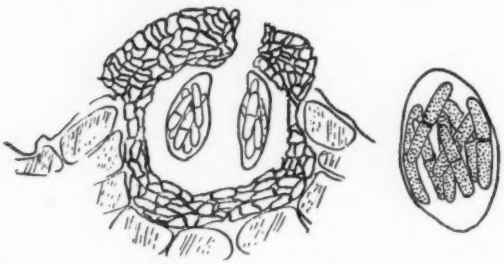

FIG. 2. *Mycosphærella gleichenia*—perithecium (× 410) and ascus with ascospores (× 850)

The spots are found on the pinnules and are ashy gray on the upper surface and brown on the lower surface. They are irregular and may sometimes involve a major portion of the pinnule. The perithecia are subepidermal and in later stages project beyond the epidermis. The wall of the perithecium is made up of two to three layers of brown cells. The asci are usually five or six in each perithecium and are obovate or elliptic with a narrowed base.

(94) *Mycosphærella rauwolfiæ* Ramakrishnan, T. S. and K., sp. nov.

Spots amphigenous, grey to brown irregular or circular, slightly depressed; perithecia amphigenous, black, minute, innate, ostiolate; asci broadly clavate, straight or, curved, hyaline, 28 × 8 μ (21–36 × 6–9), 8-spored, aparaphysate; ascospores hyaline, fusiform, 1-septate, irregularly arranged, 10·5 × 3 μ (6–12 × 3).

Maculæ amphigenæ, grisei vel brunnei coloris, irregulares, vel circulares, leniter depressæ; perithecia amphigena, nigra, minuta, innata, ostiolata; asci clavati, recti vel curvati, hyalini, octospori, aparaphysati, 28 × 8 μ (21–36 × 6–9); ascosporidia hyalina, fusiformia, 1-septata, irregulariter disposita, 10·5 × 3 μ (6–12 × 3).

On living leaves of *Rauwolfia serpentina* Benth. (Apocynaceæ); Walayar (Malabar), 24–7–1949, K. Ramakrishnan and G. Siddalinga Reddy.

FIG. 3. *Mycosphærella rauwolfiæ* asci (× 500)

The spots have at first a dull water soaked appearance. Later the centre of the spot turns brown to grey and may be dropped off. The perithecia are visible on both sides as minute black dots distributed over the entire surface.

(95) *Phæophacidium viburni* Ramakrishnan, T. S. and K., sp. nov.

Spots minute brown, circular, epiphyllous; apothecium subepidermal erumpent, opening by irregular slits, black, hypothecium thin, black; asci cylindric, narrowed towards the base, 95 × 14 $\mu$ (69–120 × 9–18), paraphysate, paraphyses simple, septate, longer than the asci, swollen at the apex; ascospores 8, spherical or elliptical, hyaline at first later turning brown, uniseriate, 12 × 8 $\mu$ (9–15 × 6–9).

Maculæ parvæ, epiphyllæ, circulares, brunnei coloris; ascomata subepidermia, erumpentia, irregulariter lacinato-dehiscentia, atra; asci cylindrici, basi attenuati, paraphysati, 95 × 14 $\mu$ (69–120 × 9–18) paraphysibus simplicibus, septatis, apice clavatis incrassatis, ascos superantibus; ascosporidia 8, globosa vel elliptica, primo hyalina, dein brunnea, 12 × 8 $\mu$ (9–15 × 6–9).

On living leaves of *Viburnum hebanthum* W. and A. (Caprifoliaceæ) Coonoor (Nilgiris), 24–6–1949, T. S. Ramakrishnan.

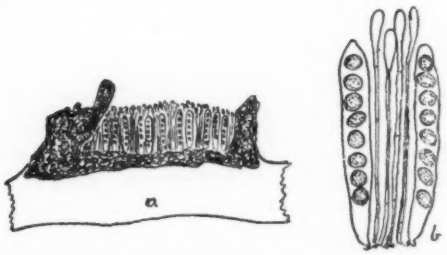


FIG. 4. *Phæophacidium viburni* (*a*) section through apothecium (× 50); (*b*) asci and paraphyses (× 250)

The apothecia originate below the epidermis. In early stages they appear as dark, convex, pulvinate formations in the middle of the spots. Very soon irregular splits are formed in the covering and in the final stages the hymenium is exposed as a circular disc. The exposed hymenium is black

in colour in the later stages. Just below the hymenial layer is the hypothecium which is made up of dark brown cells. The asci are numerous and closely packed. The paraphyses are filiform with a round or lance like swelling at the apex. The spores are elliptic or sometimes spherical. They become coloured in the later stages.

(96) *Catacaumella calamicola* Ramakrishnan, T. S. and K., sp. nov.

Stromata black, linearly arranged, hypophyllous, shining, subepidermal, multilocular; perithecia compressed globose; asci cylindric to clavate, 8-spored, aparaphysate, gelatinising quickly, 87 × 8 μ (60–105 × 6–12); ascospores hyaline, 1-celled, acicular, flattened at the base, 47 × 3 μ (36–54 × 3).

Stromata nigra, linealiter disposita, hypophylla, micantia, subepidermia, multilocularia; perithecia compresso-globosa; asci cylindrici vel clavati, octospori, aparaphysati, gelatinosi in aqua, 87 × 8 μ (60–105 × 6–12); ascosporidia hyalina, unicellata, acicularia, basi truncata, 47 × 3 μ (36–54 × 3).

On living leaves of *Calamus rotang* L., (Palmaceæ), Anamalais, 8–7–1949, T. S. Ramakrishnan.

FIG. 5. *Catacaumella calamicola* (*a*) portion of leaf showing stromata (½ nat. size) (*b*) section through stroma (× 50) (*c*) asci and ascospores (× 250)

The stromata are longitudinally arranged and appear as elongated black shining growths on the lower surface of the leaves. They are situated between the epidermis and the mesophyll. The acsi are formed from the base of the loculi. Paraphyses are absent. The ascospores are clumped irregularly inside the ascus. The wall of the ascus easily gelatinises and the loculus is filled with bundles of ascospores enveloped in the faintly visible gelatinised ascus. The spore is flattened at the base and pointed at the apex.

(97) *Plowrightia atlantiæ* Ramakrishnan, T. S. and K., sp. nov.

Spots amphigenous, minute, coalescing to form big patches, rusty brown with yellowish border; stromata mostly hypophyllous, rarely epi-

phyllous, innate, later erumpent, multilocular, locules ostiolate; asci clavate, cylindric, hyaline, aparaphysate, 8-spored, short stalked, 52 × 10 μ (39–69 × 9–12); ascospores cylindric, sometimes fusiform, 1-septate, hyaline, 20 × 3 μ (15–30 × 3).

Maculæ amphigenæ, minutæ, coalescentes, ferrugineæ; stromata prominenter hypophylla, raro epiphylla, innata, erumpentia, multilocularia, loculis ostiolatis; asci clavaticylindrici, aparaphysati, brevi pedicillati, hyalini, octospori, 52 × 10 μ (39–69 × 9–12); ascosporidia cylindrica, interdum fusiformia, 1-septata, hyalina, 20 × 3 μ (15–30 × 3).

On living leaves of *Atlantia monophylla* Corr. (Rutaceæ), Burliar (Nilgiris), 7-9-1949, T. S. Ramakrishnan.

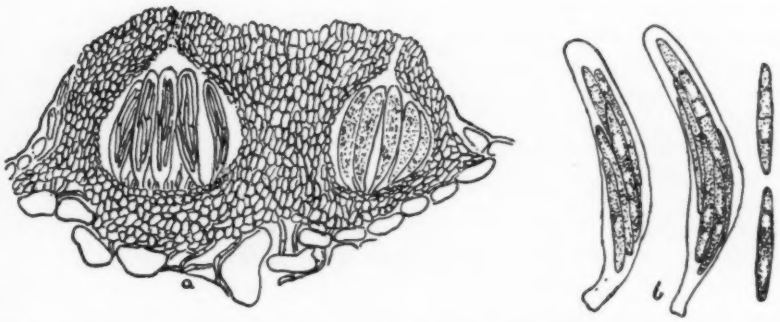


FIG. 6. *Plowrightia atlantiae* (*a*) section through stroma (× 250), (*b*) asci and ascospores (× 500)

The stromata appear in the middle of a rust coloured spot with raised edges. A number of these spots occur together and a coalescent big spot results. The central portion of the patch becomes grey in colour with age. The stroma is pseudoparenchymatous. A varying number of loculi are present in the stroma.

(98) *Uleodothis coonoorensis* Ramakrishnan, T. S. and K., sp nov.

Spots elliptic, amphigenous, dark brown; stromata amphigenous, without clypeus, subepidermal, finally erumpent, unilocular, ostiolate; asci cylindric to clavate, short stalked, hyaline, 51 × 10 μ (45–63 × 9–12); paraphysate, paraphyses filiform; ascospores 8, fusiform, 1-septate, hyaline, 14 × 3 μ (9–18 × 3).

Maculæ ellipticæ, amphigenæ, fusci brunnei coloris; stromata amphigena, subepidermia, dein erumpentia, unilocularia, clypeo nullo; loculi ostiolati; asci cylindrici vel clavati, brevi pedicellati, hyalini, 51 × 10 μ

(45–63 × 9–12), paraphysati, paraphysibus filiformibus; ascosporidia 8, fusiformia, 1-septata, hyalina, 14 × 3 $\mu$ (9–18 × 3).

On living leaves of *Phœnix* sp., (Palmaceæ), Coonoor (Nilgiris), 24–6–1949, T. S. Ramakrishnan.

FIG. 7. *Uleodothis coonoorensis*—ascus and ascospores (× 850)

A number of spots are found on a leaflet leading to its eventual drying. The stromata are subepidermal and break through the epidermis the latter being raised as a flap. Very often they are arranged in lines on both surfaces of the spot. The ascus wall is slightly thickened at the apex. The spores are arranged in two irregular rows. There are four big vacuoles in each spore and on account of this the spore may appear four-celled. But only a single septum is present in the middle of the spore. There is a slight constriction at the septum.

(99) *Puccinia peraffinis* H. Sydow.

Sydow, H. .. *Ann. Mycol.*, **11**, 1913, p. 326.

On living leaves of *Justicia simplex* D. Don. (Acanthaceæ), Coonoor 28–6–1949, T. S. Ramakrishnan.

(100) *Puccinia prainiana* Barcl.

Sydow, H. and P. *Monogr. Ured.* I, 1904, p. 635.

Syn. *Cæoma smilacinis* Barcl. *Sci. Mem. Med. Officers Army of India*, 4, **37**, 1889.

*C. smilacis* Barcl. Saccardo *Syll. Fung.*, **11**, 197.

This rust is of wide occurrence all over the Nilgiris and Wynad on *Smilax aspera* L., and *S. zeylanica* L., Hemispherical hypertrophied swellings

develop on the leaves usually projecting towards the lower surface. These are studded with deeply sunk æcia (cæomata). Sometimes the rust causes the formation of witches' broom-like growths of clusters of young slender axillary shoots bearing groups of æcia. Further, several of the berries become mummified and are profusely covered with æcia.

The telia are often hypophyllous, scattered and chocolate-brown in colour. The pedicels of the teliospores are stout and swell very much in water. The teliospores germinate as soon as they are mature producing one hyaline four-celled promycelium from each cell.

(101) *Kellermannia malabarica* Ramakrishnan, T. S. and K., sp. nov.

Spots amphigenous, of various shapes, greyish-brown surrounded by a violet margin on the upper surface; pycnidia epiphyllous, subepidermal, ostiolate, dark brown, wall thin; pycnidiospores stipitate, oblong to spindleshaped, hyaline, two-celled, setulose at apex, $11 \times 4 \cdot 5 \mu$ (8–15 × 2–6·5).

Maculæ amphigenæ, indefinitæ; pycnidia epiphylla, subepidermia, ostiolata, fusce brunnea, pariete tenui; pycnidiosporidia stipitata, oblonga, vel fusiformia, hyalina, 2-cellulata, ad apicem setulosa, $11 \times 4 \cdot 5 \mu$ (8–15 × 2–6·5).

On living leaves of *Anogeissus latifolia* Wall. (Combretaceæ) Walayar (Malabar), 24–7–1949, K. Ramakrishnan and G. Siddalinga Reddy.

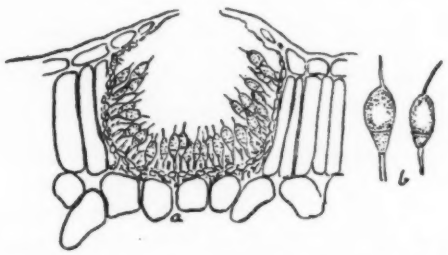


FIG. 8. *Killermania malabarica* (*a*) section through pycnidium (× 250) (*b*) spores (× 500)

A number of spots may be formed on a leaf. On the upper surface the violet margin is prominent and the central portion is slightly depressed. There is a raised ridge round the central portion. Several pycnidia are scattered on the upper surface of the spot and appear like black raised dots. The spores are two-celled the partition wall being very faint. The two cells are unequal the lower cell being smaller. A hyaline appendage is present at the apex but in scrapings it usually falls off.

(102) *Septoria dianthi* Desm. in *Ann. Sci. Nat.*, **11**, 1849, 346.

Saccardo, P. A. *Syll. Fung.*, **3**, 516, 1884.

Grove, W. B. .. *British Stem and Leaf Fungi*, **1**, p. 380, 1935.

Syn. *Ascochyta dianthi* Berk.

*A. Cookii* Mass. in *Kew Bull.*, 1907, 241.

On living leaves of *Dianthus caryophyllus* L., and other cultivated species of *Dianthus*, Sim's Park, Coonoor, 25–6–1949, T. S. Ramakrishnan.

The spores are all 1-septate. Depressed leisons are formed on the leaves and these are studded with round black pycnidia with the epidermis forming a raised white dot in the centre of each.

(103) *Septoria hydrocotyles* Desm. in *Ann. Sci. Nat.*, **17**, 1842, 109.

Saccardo, P. A. .. *Syll. Fung.*, **3**, 531, 1884.

Grove, W. B. .. *British Stem and Leaf Fungi*, I, 395, 1935.

On living leaves of *Hydrocotyle javanica* Thumb. (Umbelliferæ). Anamalais, 10–8–1949, T. S. Ramakrishnan.

(104) *Septoria phlogis* Sacc. and Speg.

Saccardo, P. A. .. *Syll. Fung.*, **3**, 533, 1884.

Grove, W. B. .. *British Stem and Leaf Fungi*, I, 395, 1935.

On living leaves of *Phlox* sp. (garden variety) (Polemoniaceæ), Sim's Park, Coonoor, 25–6–1949, T. S. Ramakrishnan.

(105) *Cercospora bombacina* Ramakrishnan, T. S. and K., sp. nov.

Spots small dark brown, amphigenous; mycelium internal; conidiophores, amphigenous, tufted, emerging through the stomata, simple, continuous, 20–25 × 4–5 $\mu$, light brown; conidia narrowly obclavate, subhyaline, to yellowish brown, curved, 1–8 septate, 54 × 3 $\mu$ (33–78 × 3).

Maculæ parvæ, amphigenæ, fusci brunnei coloris; mycelio interno; conidiophori amphigeni, fasciculati, simplices, continui, pallide brunnei, emergentes per stomata, 20–25 × 4–5 $\mu$; conidia angustato-obclavata, subhyalina vel flavo brunnea, curva, 1–8 septata, 54 × 3 $\mu$ (33–78 × 3).

On living leaves of *Bombax malabaricum* DC. (*B mbaecae*), Walayar (Malabar), 24–7–1949, K. Ramakrishnan and G. Siddalinga Reddy.

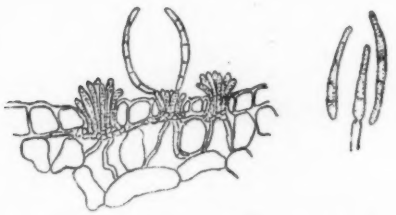

FIG. 9. *Cercospora bombacina*—conidiophores and conidia (× 250)

The spots are many on a leaf. Those on the lower surface are limited by the veins. The conidiophores are very short and project little above the surface.

(106) *Cercospora hibisci* Tracy. and Earle.

Stevens, F. L. and Solheim, W. G...*Mycologia*, **23**, 1931, 378.

Indefinite spots forming olive brown or sooty, vein limited patches are present on the lower surface of the leaf. The disease was prevalent on the Central Farm, Agricultural College, Coimbatore and in South Kanara on the leaves of *Hibiscus esculentus*. The disease is common in the monsoon months and usually on the leaves of the older plants.

(107) *Cercospora sophoræ* Ramakrishnan, T. S. and K., sp. nov.

Spots amphigenous, irregular, grey in colour, with a distinct raised margin; conidiophores in clusters appearing as black scattered dots, epiphyllous, crowded, emerging through the stomata, 2–3 septate, light olive brown, apex geniculate, 22–32 × 6·5 $\mu$; conidia curved or flexuous, 4–12 septate, 58·5 × 7 $\mu$ (43–73 × 6–9), constricted at the septa, olive brown, guttulate.

Maculæ amphigenæ, cani coloris, irregulares; conidiophori fasciculati, epiphylli, emergentes per stomata, gregarii, 2–3 septati, pallide olivaceo brunnei, apice sæpe geniculati, 22–32 × 6·5 $\mu$; conidia curva vel flexuosa, 4–12 septata, constricta ad septum, olivaceo brunnea, guttulata 58·5 × 7 $\mu$ (43–73 × 6–9).

On living leaves of *Sophora glauca* Lesch. (Papilionaceæ). Nanjanad (Nilgiris), 20–8–1949, N. V. Sundaram.

The spots are quite distinct on the upper surface being grey and surrounded by a distinct margin. Many of the spots have a distinct central irregular whitish area surrounded by a brownish margin which is visible on both sides of the leaf. The conidiophores are closely packed. Each cluster

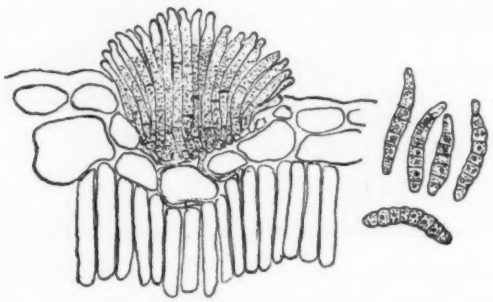

FIG. 10. *Cercospora sophoræ*—conidiophores and conidia (× 250)

originates in the substomatal space and emerges outside pushing apart the epidermal cells widely. The conidia are often bent with conspicuous constrictions at the septa. The number of septa varies.

Our thanks are due to Dr. S. K. Mukerji, Curator, Royal Botanic Gardens, Calcutta, for very kindly identifying some of the host plants and to Rev. Dr. H. Santapau of St. Xavier's College, Bombay for the latin translation.

## REFERENCES


Butler, E. J. and Bisby, G. R. .. *Fungi of India. Sci. Monogr. Coun. Agric. Res. Ind.*, 1931.

Mundkur, B. B. .. *Fungi of India*, Suppl., 1938, **1**.

Solheim, W. G. .. *Ill. Biol. Monogr.*, 1929, **12**, No. 1.

Stevens, F. L. .. *Ibid.*, 1923, **8**, No. 3.

——————— .. *Ibid.*, 1927, **11**, No. 2.

Theissen, F. and Sydow, H. .. *Ann. Mycol.*, 1915, **13**.

# ON *MYZOSTOMA STRIATA*, N. SP. FROM THE MADRAS HARBOUR

By A. I. George, M.A., M.Sc.

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(Communicated by Prof. R. Gopala Aiyar, F.A.Sc.)

## Introduction

While examining some Crinoids from the Madras Harbour, I came across two Myzostomes, one of which is a new species, and the other *M. adhærens* Remscheid (1918). Both of these were obtained from *Lamprometra palmata* Clark (1921). The only other Myzostome recorded from the Madras Coast is *M. gopalai* Subramaniam (1938) occurring on *Tropiometra*.

This species appears to be closely related to *M. fissum* Graff (1884–87) and *M. intermedium* Graff but sufficiently different from them to justify the creation of a new species *M. striata* to receive it.

*Myzostoma striata* is a very elegant form occurring attached to the dorsal surface of the arms of the Crinoid *Lamprometra palmata* Clark. There are typically six caudal appendages, nine to ten pairs of marginal cirri and very characteristic crests on the dorsal surface. It clings firmly to the pinnules and offers resistance when any attempt is made to dislodge it and crawls away with rapidity to the ventral surface or to the tips of the arms. Left in a glass dish of fresh sea-water they move their caudal appendages and parapodia vigorously and the two lateral sides fold over the ventral surface causing the hooks to interlock. They are quite helpless and lie on whichever side they are placed. Of the Crinoids examined only a few had these ectoparasites and usually only a single Myzostome was obtained from a single Crinoid. Sixteen specimens of this species were available for this study. To preserve them in an expanded condition they were kept under gentle pressure between two glass slides and killed by a stream of Bouin's fluid. They were then removed into a tube containing fresh fixative. Parapodia, cirri and suckers do not undergo much distortion. Specimens were stained with Borax carmine, cleared in oil of Cedar and mounted in Xylol balsam. Such whole mounts show very clearly the disposition of the internal organs.

*Colouration.*—The colouration of the animal is very characteristic. On an orange or orange-white ground colour is super imposed two pairs of

longitudinal violet brown colour bands. The host shows considerable variation in colour and in accordance with this the parasite shows slight changes of colour. In a typical case the dorsal side has two pairs of colour bands (Fig. 1) extending from the anterior to the posterior margin. These

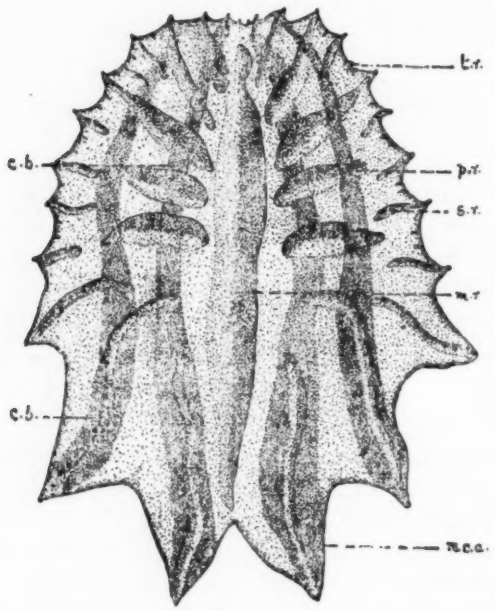


FIG. 1. Dorsal view of the animal showing the colour bands, × 20.

colour bands leave five prominent streaks of uncoloured bands. On the ventral side also there are two pairs of colour bands but they are not so prominent or broad as the dorsal ones. The inner of these are broader, shorter and extend from the inner caudal appendages to the base of the fourth pair of parapodia. The outer bands start from the middle caudal appendages and run up to the base of the first parapodia. They are considerably narrowed as they reach the anterior end and lie outside the bases of the parapodia. The tips of the caudal appendages are coloured orange. Numerous pigment spots are distributed throughout the dorsal and ventral surfaces, but more so on the ventral side. Since the arms of the *Lamprometra* also have alternating bands of white and violet brown, and the tips of the pinnules orange, the colouration of the Myzostome is of a protective nature.

*Dorsal surface.*—In the living condition the dorsal side is convex. It is thickest at the centre where the median longitudinal ridge occurs and

gradually it becomes thinner towards the margin. The dorsal surface (Fig. 1) is thrown into ridges which are very characteristic of this species. There is a median longitudinal crest running antero-posteriorly but not reaching to the very margin. It is broadest near the middle. Ridges starting from the inner and median caudal appendages end near the median longitudinal ridge. That from the former reach about one-third the length of the animal. The ridges from the middle ones stop near the centre of the disc with a sharp turn towards the central ridge. In some cases this ridge may be broken up into two. Ridges from the outer caudal appendages reach upto the middle of the outer colour bands. In addition to these there are five pairs of primary costæ radiating from the central ridge, six pairs of secondaries and six to twelve pairs of tertiaries. The first of the primaries which is the shortest starts from the second cirri, the second from the third cirri, the third from the fifth cirri, fourth from between the sixth and seventh cirri and the fifth from between the eighth and ninth cirri. The last two start from the outer colour bands and do not reach the margin. The secondary ridges start from cirri 1, 4, 6, 7, 8 and 9. They reach upto the outer border of the outer colour band. The tertiaries vary in their number from six to twelve on each side. They are confined to the marginal zone and may be mistaken for minute cirri. In one or two specimens the caudal appendages have also such short marginal crests which give a wavy appearance to the margin.

*Caudal appendages.*—This form has three pairs of caudal appendages. Of the three pairs, the outermost is the shortest and the innermost the longest. The longest caudal appendages, in fully grown specimens, reach nearly one-third the length of the body. The shortest caudal appendage may reach one-fifth the length of the body. Into these caudal appendages the alimentary diverticula and ramifications of the ovary extend and almost completely fill them. The tips of the caudal appendages are slightly swollen and in life these are used to get a grip on the pinnules. Their ventral surfaces are quite smooth while their dorsal surfaces bear the crests.

*Cirri.*—On the margin, besides these prominent caudal appendages there are nine to ten pairs of cirri. They are short triangular processes, all of them of almost the same shape and size. Typically there are nine pairs. In some cases there is a median one just opposite to the mouth opening which may not be forked. In other cases this may be separated into two distinct cirri thus giving rise to the tenth pair. Usually this pair is very minute. In no case the number of cirri exceed twenty.

*Ventral surface.*—Ventral surface (Fig. 2) is quite smooth. The five pairs of parapodia, four pairs of suckers and the male genital papillæ, mouth and cloacal openings—all these occur on the ventral surface.

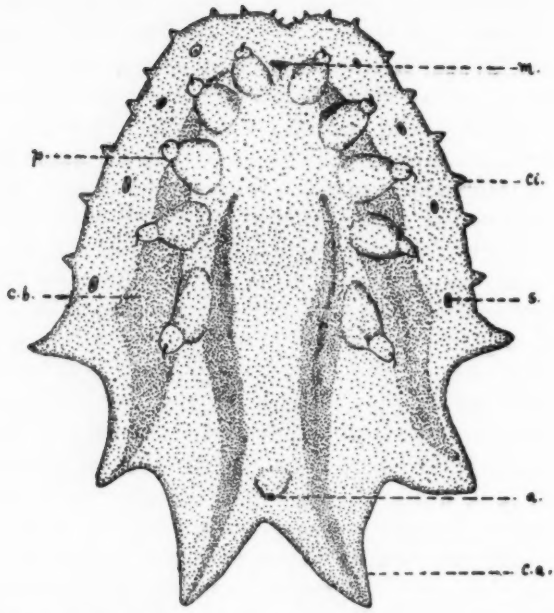


FIG. 2. Ventral view of the animal showing colour bands, parapodia, suckers, etc. × 20.

*Parapodia* (*Fig.* 3).—The parapodia are highly muscular and are situated in a broad arc on either side. Each parapodium shows division into a thick conical base and a slender elongated distal part, the basal portion gradually merging into the distal part. Supporting it is the hook apparatus. There is a strong sharply curved hook. The supporting rod—manubrium—is provided with an end plate, directing the movements of the hook. Strong muscle bands end on this end plate. In cleared specimens a succession of two or three reserve hooks are noted. In life, the parapodia are vigorously moved about in all directions and in an extended condition may reach beyond the margin of the body. The first pair lies close to, behind the mouth but the insertion of the last pair is at a considerable distance from the posterior margin of the body. The last pair is the best developed and their size decreases gradually in the preceding pairs. In position they are situated midway between the centre and the margin. Lateral to the third parapodium on either side is a male genital papilla inserted close to the base of the parapodium.

*Suckers* (*Fig.* 4).—The four pairs of suckers are rather large, and raised on muscular prominences and have complicately folded walls. The last

pair of suckers is the largest and the size of the succeeding ones decrease gradually. Suckers are situated half-way between the base of the parapodia and lateral margin.

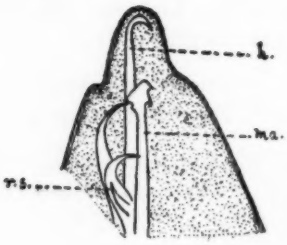

FIG. 3

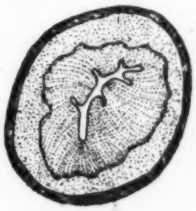

FIG. 4

FIG. 3. A parapodium showing hook, manubrium and reserve setæ. × 60.
FIG. 4. Sucker. × 100.

*Alimentary canal* (*Fig.* 5).—Mouth opening is some distance away from the margin and is circular. The mouth leads into a thick muscular slightly protrusible pharynx. In an extended condition the pharynx reaches up to the anterior margin. When extended it shows four papillæ. In preserved specimens the pharynx is always retracted and lies some distance behind the mouth opening. Pharynx is provided with a long bulbus

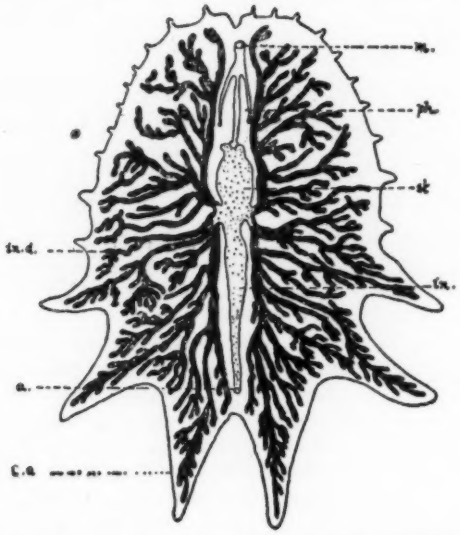

FIG. 5. The alimentary canal as seen in a cleared specimen. × 20.

musculosus. The pharynx leads into a slightly dilated stomach from which originate two pairs of alimentary diverticula. They ramify and extend throughout but mainly occupy the posterior part of the body. The walls of the intestine are thrown into folds. Cloacal opening is situated on a papilla, some distance away from the margin.

*Nerve ganglion.*—In particularly favourable specimens a large stellate ganglion with eleven pairs of nerves starting from it has been observed.

*Specific characters.*—Body shape is elliptical. Dorsal plane is faintly convex with a median longitudinal ridge and several radiating ridges. Margin is provided with ten pairs of cirri and three pairs of caudal appendages. Parapodia and suckers are prominent. Mouth and anus are ventral and subterminal. Two pairs of intestinal branches are present. Dorsal and ventral surfaces are adorned with two pairs of colour bands. A concentrated nerve ganglion is present. Occurs on *Lamprometra palmata* from the Madras Harbour.

This species differs from *M. intermedium* in that the caudal appendages have not the terminal threads. It differs from *M. fissum* in that the longitudinal furrows on the under-surface of the caudal appendages are wanting, the median longitudinal dorsal crest not reaching up to the margin and in that it has got besides the five pairs of costæ (of *M. fissum*) a number of other crests on the caudal appendages and body.

## Acknowledgment

My thanks are due to Prof. R. Gopala Aiyar, for his kindness and encouragement. This study was made at the Madras University Zoological Research Laboratory.

## References*

Atkins, D. .. "Report on the Myzostomida collected by Mr. F. A. Potts," *Proc. Zool. Soc. Lond.*, 1927.

Clark, A. H. .. "A monograph of the existing Crinoids," *Bull. U. S. Nat. Mus.* No. 82, 1921.

ch. Boulenger .. "Report on Myzostomida," *Proc. Zool. Soc.*, London, 1913.

——— .. "On a collection of Crinoids from the Indian Ocean and the Bay of Bengal," *Rec. Ind. Mus.*, 1932. 34.

von Graff, L. .. "Verzeichniss der von den United States Coast Steamers Hassler & Blake von 1867 zu 1879 gesammelten Myzostomiden," *Bull. Mus. Comp. Zool.*, 1883–85, 1885. 9.

——— .. *Das genus Myzostoma*, Leipzig, 1887.

* References indicate all the journals consulted.

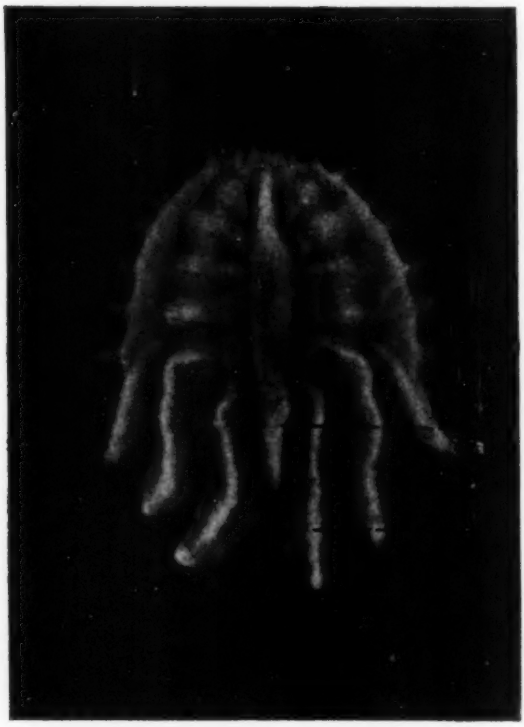


FIG. 1. Dorsal view of the animal

von Graff, L. .. "Report on the Myzostomida collected during the voyage of H. M. S. Challenger during the years 1873–76," *Rep. Chall. Exped.*, 1884. **Bd. 10.**

———— .. *Supplement Rep. Chall. Exp.*, 1887. **Bd. 10,**

Jagersten Gosta .. "Myzostomiden of Dr. Sixten Bochs Expedition," *Ark. Fur. Zool.*, 1937. **Bd. 29.**

Juta Hara and Yaichiro Okado .. "Two new species of Myzostoma," *Anot. Zool. Jap.*, 1921, **10.**

Mc Clendon, J. F. .. "New marine worms of the genus Myzostoma," *Proc. U. S. Nat. Mus.*, 1907, **32.**

Reichensperger, A. .. "Eine neue Myzostoma," *Bull. Mus. Zool. Harvard*, 1906, **43.**

Remscheid, E. .. "Beitrage zur kenntnis der Myzostomiden," *Abhandl. Senk. Nat. Ges.*, 1918, **35.**

Subramaniam, M. K. .. "On *M. gopali* from Madras Harbour," *Proc. Ind. Acad. Sci.*, 1938, **7.**


## Key to Lettering

| | | | |
|---|---|---|---|
| *a.* | anus. | *m. r.* | median dorsal ridge. |
| *c. a.* | caudal appendage. | *p.* | parapodium. |
| *ci.* | cirri. | *ph.* | pharynx. |
| *c. b.* | colour band. | *p. r.* | primary ridges. |
| *h.* | hook. | *r. s.* | reserve hooks. |
| *in.* | intestine. | *s.* | sucker. |
| *in.d.* | intestinal diverticula. | *s. r.* | secondary ridge. |
| *m.* | mouth. | *st.* | stomach. |
| *ma.* | manubrium. | *t. r.* | tertiary ridge. |

*N.B.*—Diagrams have been reduced to two-thirds the magnifications given.

# AN INVESTIGATION INTO THE NATURE OF INJURY SUSTAINED BY RICE PLANT ON ACCOUNT OF PARASITISM BY *STRIGA LUTEA* (LOUR.)

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Received May 1, 1950
(Communicated by Mr. K. Ramiah, F.A.Sc.)

## Introduction

It is a debatable point whether the baneful action of *Striga lutea* on the host-plant is confined only to its parasitic activity of depriving the nutrient materials or plant food of the host. Saunders (1933) working with *Striga lutea* parasitic on maize is of opinion that the ill-effect on the host plant is entirely due to the parasitism of *Striga*. However, it is a common observation that in a wide spaced rice plant affected by *Striga* there are quite a large number of roots and normally the number of haustorial attachments by *Striga* in this are comparatively too few to be held responsible for the high magnitude of injury done to the host plant. It, therefore, looked possible that *Striga*, besides its parasitic effect, might have a toxic action as well as any other on the rice plant. The following experiments were designed to test this possibility.

## Materials and Methods

Rice plants were grown in different cultural media such as Von-cron's solution,[1] well water and aqueous extract of soil. To these were added an aqueous extract of *Striga* root and kept in glass bottles holding 40 ml. of each along with controls as detailed below. The bottles were given an outer covering of thick paper to shut out light. Root extract of *Striga* was obtained in the form of a thick juice by macerating freshly obtained roots of the plant with sand and straining with the addition of small quantities of water. In a first set of rice plants grown in the Von-cron's solution for a month 10 ml. of this extract was added to the culture medium. In a second experiment, using ordinary well water as the culture medium the *Striga* extract was added and the above experiment repeated. In a third experiment with well water as the culture medium, root extract of *Borreria hispida*, a

[1] $KNO_3$ .. 1·00 gram. $CaSO_4$ .. ·25 gram.
$Ca_3(PO_4)_2$ .. ·25 „ $FePO_4$ .. ·25 „
$MgSO_4$ .. ·25 „ $H_2O$ .. 1000 c.c.

major weed occurring largely with *Striga* in the *modan*[2] fields, was added instead of the *Striga* extract. In a fourth experiment, the *Borreria hispida* root extract was replaced by *Leucas aspera*[3] root extract. Instead of well water, aqueous extract of the *modan* soil, aimed at approaching more near to the field conditions, was used. The soil extract was made by stirring the *modan* soil in water for 48 hours and then by decanting. Excessive quantity of soil was used to obtain a thick extract. For this experiment young rice plants about one month old were chosen. The treatments consisted of the following:

*Treatment* 1. 65 ml. of soil extract *plus* 25 ml. of well water (control).

*Treatment* 2. 65 ml. of soil extract *plus* 25 ml. of *Striga* root extract.

*Treatment* 3. 65 ml. of soil extract *plus* 25 ml. of *Leucas aspera* root extract.

In yet another experiment, the root extract of *Striga* was added to well water used as culture medium growing mature rice plants in shot blade instead of young ones.

## Experimental Results

The addition of *Striga* root extract in the first experiment produced an immediate effect in that the rice plants wilted resulting ultimately in the death of the plants while the controls continued to grow and remain healthy. In the second experiment, the result was the same as in the first experiment. In the third experiment the addition of *Borreria hispida* root extract did not kill the rice plants. In the fourth experiment the observations made, beginning from the next day of the commencement of the experiment, are set forth below and also recorded in the accompanying photograph (Fig. 1).

Observations and remarks on rice plants

| Days of observation | Treatment 1 (control) | Treatment 2 | Treatment 3 |
|---|---|---|---|
| 1st day .. | Healthy | Sign of wilting | Slight wilting |
| 2nd day .. | do | Further wilting | Partly recovered |
| 3rd day .. | do | Continued wilting | Fully recovered |
| 4th day .. | do | Totally wilted and dead | Normal |

[2] The red ferruginous soil of the slopes of hills in Malabar in which rice is grown purely as a rain-fed crop.

[3] Yet another notorious *modan* weed occurring in as large a number as *Borreria hispida* in and around the *Striga* colony.

In the last experiment, for a week the rice plants showed signs of wilting gradually until the central shoots alone remained alive. At the end of this period the plants showed signs of slow recovery. An examination of the root system revealed that all the original roots in these had ceased to function while the few new roots that came up later helped the plants to recover. But in the culture medium treated with the root extract of *Borreria hispida* and in the un-treated control, the original roots of rice were least affected and were found to function quite normally.

In a transverse section of a haustorium of *Striga* in fusion with the root of rice showing the mechanism of food absorption by the parasite from the host, it appears that the haustorial hypha bores straight into the xylem vessel injuring its structure considerably in its attempt to establish the parasitic connection with the host (Figs. 2 and 3).

## Discussion and Conclusion

The death of the rice plant in the culture solution treated with the root extract of *Striga* is evidently due to some toxic substance contained in the root extract. Although this experiment could not be repeated under field conditions the results of the experiment would seem to indicate that there is a possibility of the *Striga* root under field conditions producing similar toxic influence on the host plant. It has not been possible, however, to examine the chemical nature of the *Striga* root extract and its effect on the host. The successful resistance by the older plants of rice against *Striga* in the field has evidently some parallel to the phenomenon observed in the above experiments. The effect of *Striga* on rice may thus be both parasitic and toxic. An explanation for the difference in behaviour between the young and the old plants in contact with the *Striga* root extract in the fifth experiment may be offered by the simple assumption that the toxin, presumably an unstable chemical compound, gets inactivated after a certain period possibly being effectively lethal to plant growth only in its fresh condition of extraction and that the aged seedlings of rice were able to get over successfully the first impulse of reaction, whereas the young plants possessing less of resistance succumbed to it.

Thoday (1911) in her studies on *Cuscuta* has demonstrated that in the fusion with the sieve tubes, the invading hypha takes care to lay itself against a sieve area alongside a sieve tube in order to dissolve by hydrolysis the hyphal cell wall at the point of contact, preferring not to injure the mechanism and thereby impair the functional efficiency of the conducting vessels of the host by laying itself elsewhere on the sieve tube. The above phenomenon observed in the case of *Striga* seems to be, therefore, not exactly in

line with what has been demonstrated to be the case in *Cuscuta*. The mechanical injury done to the host vessel by the haustorial hypha in its parasitic raid appears, therefore, partly to contribute to the injury sustained by the rice plant on account of the parasite.

## Summary

The opinion held by some that the baneful action of *Striga lutea* on the host (maize) is entirely due to its depriving the host of its plant food is disputable.

In the culture experiment conducted with the young rice seedlings it appears that the death of the rice plant in the culture solution treated with the root extract of *Striga* is due to some toxic substance contained in the extract. The effect of *Striga* on rice in the field may thus be both parasitic and toxic.

From the culture experiment conducted with the young and aged rice seedlings, it is seen that the latter is able to resist the toxic effect much better than the former which perhaps partly explains why an early sown rice crop in the field is better able to resist an attack of *Striga* than a late sown one.

An explanation for the differential behaviour between the young and old rice plants in contact with *Striga* root extract in the above culture experiment is suggested.

The mechanical injury done to the host plant by the haustorial hyphæ in the establishment of parasitism appears partly to contribute to the injury sustained by the rice plant on account of this parasite. This phenomenon appears to be not strictly in line with what has been observed in the case of the parasite *Cuscuta* in which the invading hypha fuses with the host vessel in such a way as not to cause it any mechanical injury and subsequent functional degeneration.

## Acknowledgement

This work forms part of my research for the M.Sc. of the Madras University and I wish to acknowledge with thanks the kind help rendered by Dr. T. S. Sadasivan of the Madras University by his critical review of the manuscript and by making valuable suggestions towards the final get-up of the paper.

## References

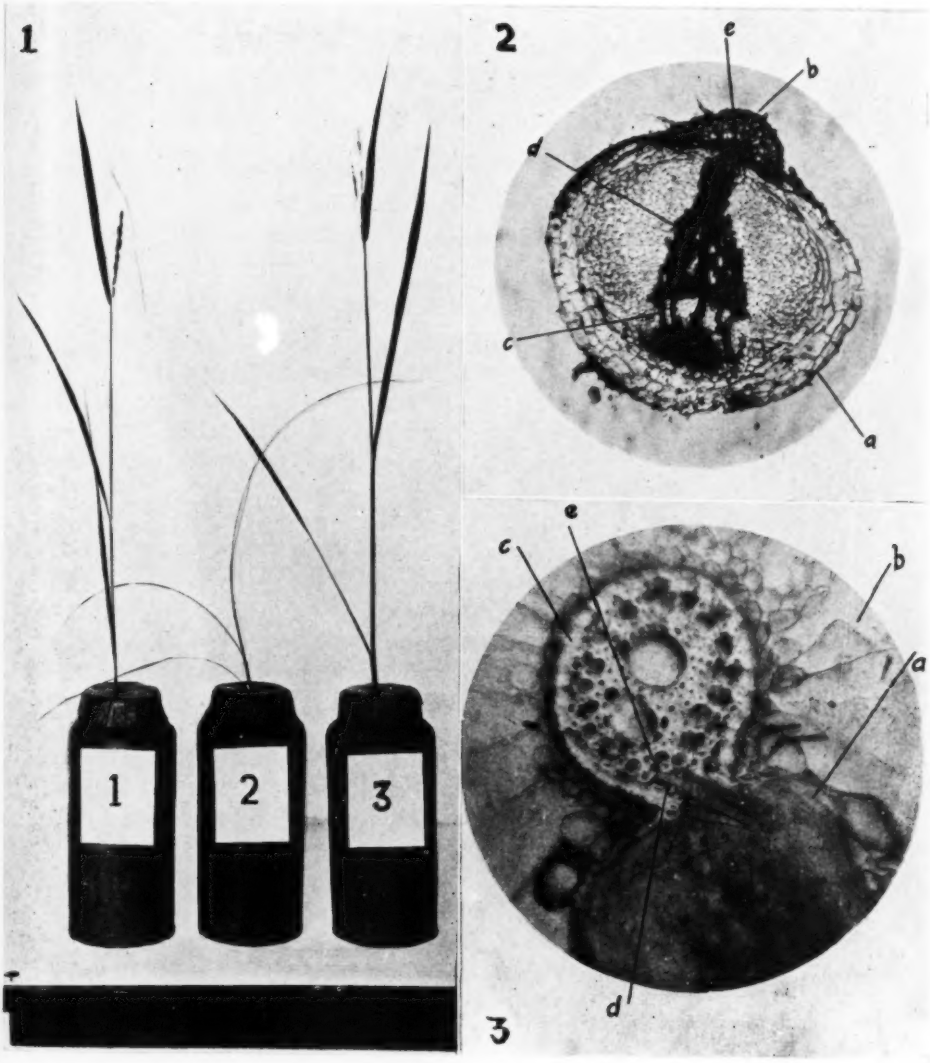
1. Saunders, A. R. .. "The Witch weed," *Dep. Agri. Union*, S. Africa, 1930, 5, 267–71.
2. Thoday, Mary, G. .. "On the Histological Relations between *Cuscuta* and its Host," *Ann. Bot.*, 1911, 25, Part 2, 655–82.

## Description of Plate

Fig. 1. Experiment to show the toxic effect of *Striga* on rice. The photograph shows rice growing in culture medium contained in covered glass bottles. Nos. 1, 2 and 3 represent the same culture medium treated with ordinary well water, root extract of *Striga* and the root extract of *Leucas aspera* respectively. Note the wilting of rice plant in No. 2.

Fig. 2. Microphotograph of the transverse section of a haustorium in fusion with the root of rice showing the mechanism of food absorption by the parasite from the host root. *a*. Section of the haustorium; *b*. Section of the rice root; *c*. Main haustorial vessels as having branched out from the conducting vessels of the *Striga* root; *d*. Sub-branches from the main vessels of the haustorium; *e*. Fusion of the haustorial vessels with the conducting vessels of the host root.

Fig. 3. A highly magnified photo-micrograph of the transverse section of a haustorium that had fused with the rice root after the *Striga* had emerged from the ground. The photograph shows the fusion of haustorial hypha with the xylem vessel of the host root. *a*. Tip end of the penetrating haustorium; *b*. Cortex of the rice root; *c*. Stele of the rice root; *d*. Haustorial hypha whose pre-hensile tip has bored into the xylem; *e*. of the host × 265.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# CHROMOSOME NUMBERS IN SOME SPECIES OF *DICHANTHIUM* Willemet, AND *BOTHRIOCHLOA* O. Ktze.

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Received March 13, 1950

(Communicated by Professor L. S. S. Kumar, F.A.SC.)

Cytological examination of three species of *Dichanthium*, namely *D. annulatum* Stapf., *D. caricosum* A. Camus and *D. nodosum* Willem., and one species of *Bothriochloa*, namely *B. intermedia* (Br.) A. Camus, was undertaken with a view to determine the meiotic and somatic chromosome numbers in these species and to compare with the findings of earlier workers. This information is of basic importance for carrying out any work on the improvement of grasses for their fodder value.

## Previous Work

Karper and Chisholm (1936) found 20 bivalents at diakinesis in the pollen mother-cells of *D. annulatum*. Janaki Ammal (1945) confirmed this number (Unpublished). Krishnaswamy (1939) has determined 40 as the somatic chromosome number in *D. annulatum* and 50 in *D. caricosum*. The chromosome number as determined by Krishnaswamy (1939) has been questioned by Janaki Ammal (1945). In *B. intermedia*, Avdulov (1931) found 60 somatic chromosomes.

## Material and Method

The material obtained locally and from other places (*vide* Table I) was used for the determination of chromosome numbers. Fixing of root-tips was done from June to October as from November onwards the culms produced few and very thin roots. As the usual methods of preparing root-tips for chromosome counts proved unsatisfactory because of the relative length and tendency for overlapping of the somatic chromosomes, Hill and Myers (1945) method of cold treatment was employed with suitable modifications. Exposure of roots to 8° C. for 24 hours resulted in satisfactory contraction of the chromosomes which was necessary for precision in making counts from metaphase plates. After fixing for 24 hours, root-tips were washed in distilled water and placed in 50% alcohol for one hour. They were then passed through alcohol series to paraffin as suggested by Randolph (1935). Sections were cut at 10$\mu$ and stained in Iodine-gentian violet.

Acetocarmine smears were prepared for the purpose of meiotic chromosome counts. Extremely few plates showed polar views due to the fact that the P.M.C.'s were more long than broad.

## Observations

The chromosome numbers of the different species examined are tabulated in Table I.

### Table I

*Chromosome number of some species of* Dichanthium *and* Bothriochloa

| Species & type | Place | Chromosome Numbers | | Remarks |
|---|---|---|---|---|
| | | Meiotic | Somatic | |
| *D. annulatum* | | | | |
| (*a*) Bushy | Poona | 20 | 40 | *Vide* Figs. 1, 2 & 9 |
| (*b*) Spreading | Poona | 20 | 40 | |
| *D. annulatum* | Nagpur | .. | 40 | |
| ,, | Cawnpur | .. | 40 | |
| *D. caricosum* | Surat | 20 | 40 | *Vide* Figs. 3, 4 & 10 |
| ,, | Ahmedabad (Jaska) | 20 | 40 | |
| ,, | Bailhongal | 20 | 40 | |
| *D. nodosum* | Jalgaon | 20 | 40 | *Vide* Fig. 5 |
| ,, | Poona | 20 | 40 | |
| *B. intermedia* | | | | |
| (*a*) Large | Poona | .. | 60 | *Vide* Fig. 7 |
| (*b*) Small | Poona | 20 | 40 | *Vide* Figs. 8 & 6 |

All the plants of *D. annulatum* from various localities showed the meiotic and somatic chromosome numbers of 20 and 40 respectively. This confirms the observations of the previous workers and there appears to be no polyploidy.

The meiotic and somatic numbers observed in *D. caricosum* collected from various localities were 20 and 40 respectively. This somatic number differs from that reported by Krishnaswamy (1939). The clarity of the preparations in the present study leaves no doubt as to the correctness of the number reported here. The shape and size of the somatic chromosomes in *D. annulatum* and *D. caricosum* are very similar.

Plants with 40 and 60 as their somatic chromosome numbers were observed in *B. intermedia*. Plants having 60 chromosomes were nearly one and half times as large as the plants with 40 somatic chromosomes. The comparative data with reference to the two chromosome types are given below.

TABLE II

*Comparative data in respect of the characters of* 40 *and* 60 *chromosome types of* B. intermedia

| Ser. No. | Characters | 40 Chromosometype | 60 chromosome type |
|---|---|---|---|
| 1 | Average height | 77 cm. | 110 cm. |
| 2 | Average spread | 102 cm. | 145 cm. |
| 3 | Average number of seeds per panicle, under open pollination | 103·9 | 18·8 |
| 4 | Average number of seeds per panicle, under paper bag | 85·6 | 15·6 |
| 5 | Average length of the third leaf from the base of the panicle | 12·12 cm. | 18·14 cm. |
| 6 | Average breadth of the third leaf from the base of the panicle | 0·45 cm. | 0·60 cm. |
| 7 | Dry weight of 20 leaves (per plant) consisting of 3rd leaves from the base of each panicle | 0·40 gm. | 0·95 gm. |
| 8 | Length of nodal hairs at first node from top | 0·88 cms. | 0·13 cm. |
| 9 | Average length of main rachis | 2·79 cm. | 3·85 cm. |
| 10 | Average length of peduncle from base of panicle to first node | 21·87 cm. | 28·51 cm. |
| 11 | Average length of first spike from the base of panicle | 5·7 cm. | 6·8 cm. |
| 12 | Average breadth of the first spike from the base of panicle | 0·11 cm. | 0·13 cm. |
| 13 | Number of plants obtained by sowing 100 seeds | 20 | 13 |
| 14 | Date of harvest for hay | December | February |

The size of the chromosomes in the 60 chromosome type is smaller than that in the 40 chromosome type. This agrees with Avdulov's (1931) observation that chromosome size decreases with increase in polyploidy.

In *D. nodosum* Willem., the somatic chromosome number observed was 40. This number is being reported for the first time.

## SUMMARY

1. Cytological examination of *D. annulatum, D. caricosum, D. nodosum* and *B. intermedia* was undertaken.

2. Meiotic and somatic chromosome numbers observed namely $n = 20$ and $2n = 40$ in *D. annulatum* are in confirmation with the observations of the previous workers.

3. Meiotic and somatic chromosome numbers in *D. caricosum* have been observed as 20 and 40 respectively for the first time as the somatic number reported by Krishnaswamy (1939) does not agree with the number reported here.

4. The somatic chromosome number in *D. nodosum* is $2n = 40$. This is being reported for the first time.

5. No polyploidy was observed in the forms of the three species of *Dichanthium* studied.

6. In *B. intermedia* 40 chromosome type is being reported for the first time and the existence of 60 chromosome type is confirmed.

7. The existence of polyploidy has been observed in *B. intermedia* and the increase in chromosome number is associated with the increase in size and other characters.

The author is greatly indebted to Professor L. S. S. Kumar, for his guidance and encouragement during the course of this work.

## References

1. Avdulov, N. P. .. *Bull. Appl. Bot. Genet. etc., Suppl.*, 1931, 43 & 44.
2. Darlington, C. D. and Janaki Ammal *Chromosome Atlas of Cultivated Plants*, George Allen and Unwin Ltd. London, 1945, 327–28.
3. Hill, H. D. and Myers, W. M. .. "A schedule including cold treatment to facilitate somatic chromosome counts in certain forage grasses," *Stain Techno*, 1945, **20**, 89–92.
4. Karper, R. E. and Chisholm, A. T. "Chromosome number in Sorghum," *Amer. Jour. Bot.*, 1936, **23**, 369–74 illus.
5. Krishnaswamy, N. .. "Untersuchungen zur Cytologie und Systematik der Gramineen," *Beih. Bot. Zbl.*, 1939, **60**, 1.
6. Randolph, L. F. .. "A new fixing fluid and a revised schedule for the paraffin method in plant cytology," *Stain Techno*, 1935, **10**, 95–96.

## Explanation of Figures

(A) *Photographs of Camera lucida drawings reduced by 1/10ths*

Fig. 2. Somatic metaphase of *D. annulatum*. $2n = 40$, Magnification × 4,000.
Fig. 4. Somatic metaphase of *D. caricosum*. $2n = 40$, Magnification × 4,000.
Fig. 5. Somatic metaphase of *D. nodosum*. $2n = 40$, Magnification × 4,000.
Fig. 7. Somatic metaphase of *B. intermedia*. $2n = 60$, Magnification × 4,000.
Fig. 8. Somatic metaphase of *B. intermedia*. $2n = 40$, Magnification × 4,000.
Fig. 9. Meiotic metaphase of *D. annulatum*. $n = 20$, Magnification × 2,000.
Fig. 10. Meiotic metaphase of *D. caricosum*. $n = 20$, Magnification × 1,300.

(B) *Photomicrographs.*

Fig. 1. Somatic metaphase of *D. annulatum*. $2n = 40$, Magnification × 1,750.
Fig. 3. Somatic metaphase of *D. caricosum*. $2n = 40$, Magnification × 3,300.

(C) *Photograph of Bothriochloa intermedia.*

Fig. 6. *Bothriochloa intermedia*, 40 and 60 chromosome types.

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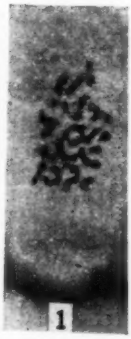
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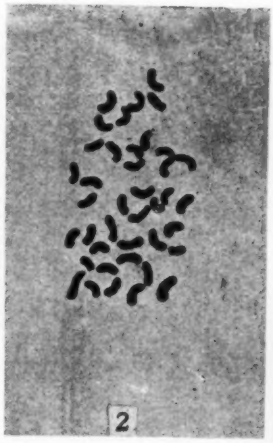
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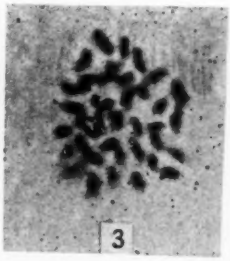
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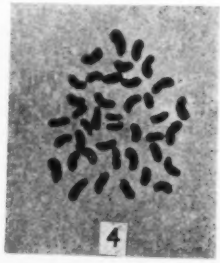
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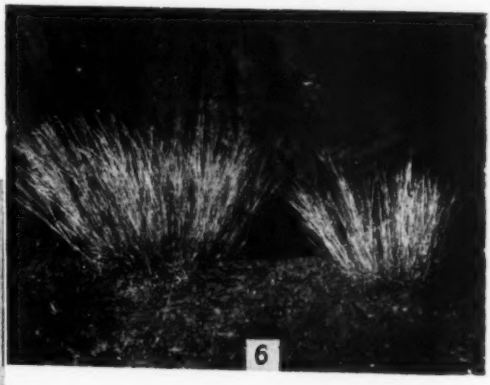
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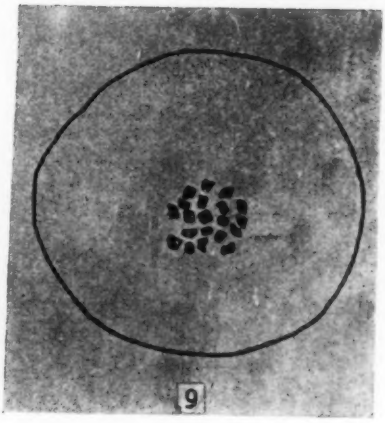
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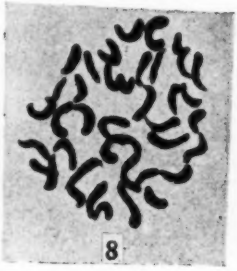
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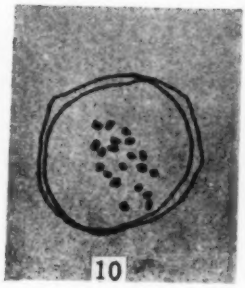
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# BIOSYNTHESIS OF VITAMIN C DURING GERMINATION

## II. Concomitant Changes in Ascorbic Acid Oxidase, Glutathione, Nicotinic Acid, Amylase, Reducing Sugars, Phosphatase and Free Phosphates

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Various attempts have been made to correlate the biosynthesis of vitamin C with one or more factors in the metabolic functioning of animals and plants. The relationship of ascorbic acid to dehydroascorbic acid, ascorbic acid oxidase and dehydroascorbic acid reductase is apparent and has been emphasized by Crook,[1] Crook and Morgan[2] and others. Among other changes having a possible bearing on vitamin C are those relating to the sugars as well as the sugar acids, glutathione and vitamins of the B group. However, earlier studies on these related changes were largely haphazard and have lacked generalization.[3] The following observations refer to a study of the changes during germination in certain constituents that were thought likely to influence the biogenesis of the vitamin. The changes in ascorbic acid and dehydroascorbic acid have been presented earlier,[4] and have been followed here by a corresponding study of the changes in ascorbic acid oxidase, glutathione, nicotinic acid, reducing sugars, amylase, phosphatase and free inorganic phosphates.

It has been shown earlier[4] that germination in the dark results in pronounced ascorbic acid synthesis as compared to other treatments studied. It is reasonable to assume that, under this condition, the related changes would also be equally marked. Hence, the major part of the following studies are confined to a comparison of the changes in various related factors as a result of germination normally and in the dark.

### Experimental

The procedures followed for seed germination, etc., were as described before.[4]

### The Changes in Ascorbic Acid Oxidase during Germination

*Determination of ascorbic acid oxidase activity.*—A weighed quantity (1–3 gm.) of the material was extracted by grinding with water three times and the combined extract was made to 100 c.c. A 5 c.c. aliquot of this extract

was added to 5 c.c. of a standard (80 mgm. per cent.) solution of ascorbic acid and kept at 37·5° C. for one hour. The enzyme was inactivated at the end of this period by addition of 10 c.c. of a 10 per cent. solution of oxalic acid and the ascorbic acid remaining was determined by titration against the standard dye solution (*a* mgm.). A blank, made up of 5 c.c. ascorbic acid solution and 5 c.c. water, was similarly kept and titrated for ascorbic acid after acidification (*b* mgm.). Another blank consisted of 5 c.c. ascorbic acid solution and 5 c.c. of the heated extract (100° C. for 10 minutes) (*c* mgm.). This last determination was considered necessary in view of the possible presence of any protective factor for ascorbic acid in the extract under study (*cf.* [5]).

Enzyme activity will be given by the figure $(c-a)$ while $(c-b)$ would indicate protection, if any, afforded by the extract to the vitamin against normal destruction in aqueous solution. It was observed that frequently *b* and *c* were the same; besides, trials with and without phosphate-buffered (pH 6) extracts did not show appreciable differences in oxidase activity and hence the use of buffered extracts was made only occasionally to check results.

The activity of ascorbic acid oxidase has been given in gm. of ascorbic acid that would be oxidized in one hour at 37·5° C. by the enzyme present in 100 gm. of dry material.

*Effect of $Mn^{\cdot\cdot}$ treatment on ascorbic acid oxidase.*—In continuation of earlier observations on the effect of $Mn^{\cdot\cdot}$ treatment of the seed on ascorbic acid and dehydroascorbic acid formation during germination, ascorbic acid oxidase activity was determined and comparisons made in its variations from day to day during germination of *mung* seeds, untreated as well as treated with 50 p.p.m. of $Mn^{\cdot\cdot}$ (Table 1).

TABLE I

*Changes in oxidase activity in relation to $Mn^{\cdot\cdot}$*

| Day of germination | Ascorbic acid oxidase activity | | Per cent. difference |
|---|---|---|---|
| | Untreated | $Mn^{\cdot\cdot}$ treated | |
| 1 | nil | nil | nil |
| 2 | 0·076 | 0·113 | +45·6 |
| 3 | 0·537 | 0·832 | +49·4 |
| 4 | 1·030 | 1·104 | + 7·2 |
| 5 | 1·983 | 1·868 | − 4·7 |
| 6 | 2·936 | 2·415 | −17·7 |
| 7 | 3·258 | 2·865 | −12·1 |
| 8 | 5·912 | 5·913 | nil |

With untreated as well as $Mn^{++}$ treated seeds, there was no oxidase present on first day of germination and on the second day only a slight activity was observable. Thereafter, there was a steady increase in oxidase activity until, on the eighth day, the day on which the withering of the seedling was first noticeable, it was nearly double that of the previous day. In general, there was no appreciable difference in the activity of the enzyme as a result of $Mn^{++}$ treatment except on the second and third days when there was a pronounced increase in the treated seedlings.

Oxidase activity of the leaves, cotyledons and stems of the treated seeds was determined separately on the fifth day of germination and compared with the activity of the corresponding parts of untreated seeds (Table II).

TABLE II

*Distribution of oxidase in seedlings and effect of $Mn^{++}$ treatment*

| | Ascorbic acid oxidase activity | | Per cent. difference |
|---|---|---|---|
| | Untreated | $Mn^{++}$ treated | |
| Leaves .. | 15·55 | 15·03 | −3·344 |
| Cotyledons .. | 3·204 | 3·252 | +1·498 |
| Stems .. | 10·140 | 10·259 | +1·174 |

The distribution of the activity in the different parts of the treated seedling was more or less similar in both cases.

The effect, if any, of the addition of $Mn^{++}$ to an extract of seedlings on its oxidase activity was also ascertained. To 5 c.c. of 80 mgm. per cent. solution of ascorbic acid were added, 5 c.c. of aqueous seedling extract and 5 c.c. of standard (150 p.p.m. $Mn^{++}$) manganese sulphate solution; the mixture was let stand for one hour at 37·5° C. Parallel trials were carried out with mixtures of (i) 5 c.c. ascorbic acid solution and 10 c.c. water; (ii) 5 c.c. ascorbic acid solution, 5 c.c. water and 5 c.c. manganese sulphate solution; (iii) 5 c.c. ascorbic acid solution, 5 c.c. unheated extract and 5 c.c. water; and (iv) 5 c.c. ascorbic acid solution, 5 c.c. heated extract and 5 c.c. water.

The unoxidized ascorbic acid was determined in each case after addition of 10 c.c. of 10 per cent. oxalic acid at the end of one hour. If this is connoted respectively *a*, *b*, *c*, *d* and *e* mgm., in the various mixtures, then oxidation caused by $Mn^{++}$ would be $(b-c)$ mgm., that caused by the extract $(b-d)$ mgm. and that caused on addition of $Mn^{++}$ to the extract $(b-a)$ mgm. The enhancement in oxidation caused by the addition of $Mn^{++}$ to the extract would

then be $(b-a) - [(b-d) + (b-c)]$. Percentage oxidation of ascorbic acid caused by $Mn^{\cdot\cdot}$ in solution will be $\frac{(b-c)}{b} \times 100$ and the percentage increase in the ascorbic acid oxidase activity by the addition of $Mn^{\cdot\cdot}$ to the extract will be $\frac{c+d-a-b}{b-d} \times 100$.

It was observed in a typical experiment, that ascorbic acid oxidation caused by $Mn^{\cdot\cdot}$ (50 p.p.m.) was 9·1 per cent. and the activation of ascorbic acid oxidase caused by $Mn^{\cdot\cdot}$ was 12·84 per cent. on third day of germination and 19·32 per cent. on fifth day of germination of the untreated seeds. A similar addition of $Mg^{\cdot\cdot}$ in the same concentration caused 3·85 per cent. oxidation of ascorbic acid, and 0·61 per cent. enhancement of the enzymic oxidation of ascorbic acid by untreated seedlings at third day of germination and 0·70 per cent. on fifth day of germination.

*Effect of aqueous extract of germinating seeds on activity of ascorbic acid oxidase of cucumber.*—It has been shown earlier that no enzymic oxidation of ascorbic acid was caused by extracts of germinated seeds on the first day and that only a slight activity was present on second day. It was observed however, that if these extracts were added to an extract of cucumber, a high enhancement of its oxidative power was observed. The relavent experiments were carried out as follows:—

Cucumber rind (about 1 gm.) and germinated *mung* (about 15 gm.) were each extracted with water in the usual way and the extracts were made to 100 c.c. Reaction mixtures consisted of 5 c.c. of 80 mgm. per cent. solution of ascorbic acid with (i) 6 c.c. water, (ii) 5 c.c. water + 1 c.c. cucumber extract, (iii) 5 c.c. *mung* extract + 1 c.c. water (iv) 5 c.c. *mung* extract (heated) + 1 c.c. water, (v) 5 c.c. *mung* extract + 1 c.c. cucumber extract, (vi) 5 c.c. *mung* extract (heated) + 1 c.c. cucumber extract. Reaction, in each case, was allowed to proceed for one hour at 37·5° C. If $a$, $b$, $c$, $d$, $e$ and $f$ denote respectively milligrams of residual ascorbic acid determined at end of one hour after acidification in each of the above cases, then oxidation of ascorbic acid due to:—

the cucumber extract would be $(a - b)$ mgm.,
the *mung* extract—$(a - c)$ mgm.,
the *mung* extract (heated)—$(a - d)$ mgm.,
the mixture of cucumber extract and *mung* extract—$(a - e)$ mgm.,
the mixture of cucumber extract and *mung* extract (heated)—$(a - f)$ mgm.

Therefore, enhancement of the enzymic oxidation of ascorbic acid by the cucumber extract due to addition of 5 c.c. *mung* extract would be $(a - e) - [(a - b) + (a - c)]$. Similarly, enhancement due to the addition of 5 c.c *mung* extract (heated) would be $(a - f) - [(a - b) + (a - d)]$.

The activation of cucumber extract oxidation, that would be caused by the extract of 1 gm. *mnug* (dry weight) could be calculated. This enhancement on the first day of germination was, in a typical protocol, 2·07 times, by the unheated *mung* extract and 1·87 times by the heated extract; on the second day of germination the corresponding values were 1·131 and 0·989. No enhancement was caused by the extract of *mung* sampled on subsequent days of germination.

*Ascorbic Acid Oxidation by Plant Extracts in relation to their copper contents.*—A comparison was made of the oxidase activity of plant extracts with the oxidising power of their ash and of the total copper in the ash. Ionic copper was determined colorimetrically using sodium-di-ethyl carbamate and the Klett-Summerson photo-colorimeter with filter 54 in position. In a typical experiment, 1 c.c. of 3 per cent. cucumber rind extract oxidised 3·45 mgm. of vitamin C at the end of one hour, there being no oxidation by the heated extract. The copper content of its ash was 640 $\gamma$ and its equivalent of ionic copper, as hydrated copper sulphate solution, oxidised 0·71 mgm. of ascorbic acid in the same period. The aqueous extract of the ash oxidised only 0·11 mgm. of the vitamin. Thus, the proportion of vitamin C oxidation due to copper in the total oxidation caused by the extract is about 1 : 5 in the absence of the protective factor which, if present, would further reduce $Cu^{\cdot\cdot}$ oxidation.

*Protection of ascorbic acid against oxidation by a thermostable factor in seed extract.*—Krishnamurthy and Giri[5] claimed to have separated, from juice of certain vegetables, a thermostable factor protecting ascorbic acid from oxidation; however, this protection was offered only against non-enzymic oxidation both in presence and absence of added ionic copper. The following method was adopted to determine the activity of the protective factor in seedling extracts.

To 5 c.c. of 80 mgm. per cent. solution of ascorbic acid were added (i) 7 c.c. water, (ii) 5 c.c. water + 2 c.c. $Cu^{\cdot\cdot}$ solution (10 mgm. of hydrated copper sulphate dissolved in 100 c.c. water), (iii) 2 c.c. water + 5 c.c. heated extract and (iv) 2 c.c. $Cu^{\cdot\cdot}$ solution + 5 c.c. heated extract. Ascorbic acid remaining in each case, after one hour's standing at 37·5° C. was determined in the usual way. If *a*, *b*, *c* and *d* denote in mgm. the respective values, then oxidation of ascorbic acid caused by

$Cu^{++}$ solution would be $(a - b)$ mgm.,
heated extract $(a - c)$ mgm.,
the mixture of $Cu^{++}$ solution and heated extract $(a - d)$ mgm.

and the protection caused by the heated extract against $Cu^{++}$ oxidation will be $[(a - b) + (a - c)] - (a - d)$ mgm.

In this way, it was observed that 5 c.c. of the heated extract of about 10–15 gm. of seedlings (*mung*), one to five-day old, inhibited completely the oxidation of the standard solution of ascorbic acid by 2 c.c. of $Cu^{++}$ solution. Both heated and unheated extracts of overnight soaked seedlings inhibited completely the $Cu^{++}$ oxidation of ascorbic acid. However, if the $Cu^{++}$ solution in the above experiment was replaced by unheated extract of seedlings or of cucumber containing the oxidase, no protection by the heated extract against the enzymic oxidation could be detected at any time during the germination period. In fact, as stated already, an enhancement in the enzymic oxidation was caused in some cases, especially on first and second day of germination, by the addition of the heated extract.

It is thus interesting that the same extract, while protecting ascorbic acid against copper oxidation, is not merely ineffective towards enzymic oxidation, but sometimes acts as an activator. Stotz, *et al.*[17] observed that an association of copper and gelatin, or of copper and albumin behaved in the same way as ascorbic acid oxidase. To explain the inactivation of the catalytic activity, these authors postulated the removal of copper from solution by precipitation, or its conversion to some inactive association compound. Since the results presented here seemed to be in disagreement with the supposed role of ionic copper in the ascorbic acid oxidase, the observations were extended further.

An attempt was made to inactivate $Cu^{++}$ in copper sulphate solution, by heating it with *mung* extract. 10 c.c. of a 1 : 10 *mung* extract were heated with 4 c.c. of standard copper sulphate solution at 100° C. for 15 minutes, 7 c.c. of this mixture were added to 5 c.c. standard ascorbic acid solution; and the vitamin remaining after one hour standing at 37·5° C. was determined. The value for the residual ascorbic acid was compared with the ascorbic acid remaining in another reaction mixture of 5 c.c. of standard ascorbic acid solution + 5 c.c. heated extract + 2 c.c. copper sulphate solution after one hour standing at 37· 5° C. There was no difference in the two values for the residual vitamin. For example, the experiments with fourth day *mung* extract showed that, in both cases, 1·31 mgm. of ascorbic acid

had been oxidised in one hour, and the inhibition in $Cu^{\cdot\cdot}$ oxidation was 44·3 per cent. Had there been even a partial inactivation of $Cu^{\cdot\cdot}$ action by heat treatment in presence of *mung* extract (as the oxidase is inactivated), the oxidation in the former would have heen less than that caused by a mixture of heated extract + $Cu^{\cdot\cdot}$ solution.

Experiments were also carried out to see the combined effect of ascorbic acid oxidase and copper sulphate solution.

An aqueous extract of seedlings (about 10 gm.) was prepared in the usual way. If the residual ascorbic acid in the reaction mixtures of 5 c.c. standard ascorbic acid solution with (i) 7 c.c. water, (ii) 2 c.c. copper sulphate solution + 5 c.c. water, (iii) 2 c.c. water and 5 c.c. unheated extract, (iv) 2 c.c. copper sulphate solution + 5 c.c. unheated extract, be denoted as $a$, $b$, $c$ and $d$ mgm. respectively, the oxidation caused

by $Cu^{\cdot\cdot}$ would be $(a - b)$ mgm.,

by extract $(a - c)$ mgm.,

by the mixture of $Cu^{\cdot\cdot}$ solution and extract $(a - d)$ mgm.

The sum of the oxidations caused by the $Cu^{\cdot\cdot}$ and the extract separately would be $(a - b) + (a - c)$. The value $(a - d)$ was found to be less than that of $(a - b) + (a - c)$.

Thus the percentage inhibition over total oxidation will be

$$\frac{[(a - b) + (a - c)] - (a - d)}{(a - b) + (a - c)} \times 100$$

This inhibition over total oxidation was found to be 35·1 per cent. on fourth day and 29·5 per cent. on sixth day. An extract of cucumber behaved in the same way as the seedling extract and there was an inhibition of 41·2 per cent.

This behaviour of vegetable extracts in respect of copper activity on ascorbic acid would suggest that the thermostable ingredient of the extract, inhibiting specifically the copper oxidation, is active even in presence of ascorbic acid oxidase.

*Effect of cold treatment.*—The oxidase activities of pea seeds (black variety) soaked and germinated normally were compared with those of corresponding lots steeped for 24 hours at 5° C. prior to germination (Table III).

Temperature treated seeds showed significantly less activity than untreated seeds at all times during germination. Thus, increase in ascorbic

TABLE III

*Effect of cold-steeping of seeds on oxidase activity of* pea *seedlings* (Pisum arvense)

| Day of germination | Ascorbic acid oxidase activity | | Per cent. difference |
|---|---|---|---|
| | Untreated seeds | Cold treated seeds | |
| 1 | nil | nil | nil |
| 2 | 0·184 | 0·138 | −24·8 |
| 3 | 0·570 | 0·547 | − 4·0 |
| 4 | 1·489 | 1·209 | −18·8 |
| 5 | 2·103 | 1·813 | −13·8 |
| 6 | 2·435 | 1·946 | −20·1 |
| 7 | 2·808 | 2·117 | −24·6 |
| 8 | 3·292 | 2·448 | −25·6 |
| 9 | 3·701 | 2·790 | −24·6 |

acid synthesis[4] is accompanied by a decrease in dehydroascorbic acid and in ascorbic acid oxidase.

Observations in respect of distribution, between the different parts of the seedlings, of ascorbic acid, and of dehydroascorbic acid had shown that cold treatment brought about a distinct difference as compared to untreated seeds. The oxidase activities in (*a*) leaves, (*b*) cotyledons and (*c*) stems of *mung* seeds steeped at 5° C. for 24 hours were measured separately on the fifth day of germination and compared with corresponding untreated seedling parts (Table IV).

TABLE IV

*Effect of cold treatment on the distribution of oxidase in* Mung *seedling*

| | Oxidase activity | | Per cent. difference |
|---|---|---|---|
| | Untreated | Cold treated | |
| Leaves .. | 15· 55 | 16· 21 | + 4·24 |
| Cotyledons .. | 3·204 | 2·507 | −21·76 |
| Stems .. | 10·140 | 9·033 | −10·91 |

The activity of the enzyme in the leaves of the treated seeds was higher than in the leaves of the untreated seeds, while the reverse was the case with cotyledons and stalk. The high oxidase activity of leaves accompanied a similar high content of vitamin C though the amount of dehydroascorbic acid in leaves was proportionately insignificant.

*Effect of germination in the dark on oxidase activity of seedlings.*—Ascorbic acid oxidase activities of *mung* seeds, germinated in the dark, were determined on alternate days during germination for five days and were compared with corresponding controls (Table V).

TABLE V

*Oxidase activity in* Mung *seeds germinated in the dark*

| Day of germination | Oxidase activity | | Per cent. difference |
|---|---|---|---|
| | Diffuse light | Darkness | |
| 1 | nil | nil | nil |
| 3 | 2·184 | 1·806 | −17·31 |
| 5 | 4·143 | 3·118 | −24·72 |

As with cold treated pea seeds (Table III), germination in dark resulted in appreciable lowering of oxidase activity when compared with normally germinated seeds.

Data on the activity of the enzyme, measured separately in (*a*) leaves, (*b*) cotyledons and (*c*) stems of seedlings germinated in dark, on fifth day of germination are compared in Table VI with those for untreated seedlings.

TABLE VI

*Effect of germination in dark on the distribution of oxidase in seedlings*

| | Oxidase activity | | Per cent. difference |
|---|---|---|---|
| | Untreated | Germinated in dark | |
| Leaves .. | 15· 55 | 17· 69 | +13·76 |
| Cotyledons .. | 3·204 | 2·855 | −10·90 |
| Stems .. | 10·140 | 7·702 | −24·04 |

As with seeds pretreated by steeping in the cold, oxidase activity in treated seedlings was more in the leaves and less in the cotyledons and stems when compared to normally germinated seedlings.

HEXOSE DEHYDROGENASE

The existence of a hexose dehydrogenase in germinating seeds, bringing about the aerobic and anaerobic oxidation of glucose, galactose and mannose to ascorbic acid has been reported by Rudra,[6]

1 c.c. enzyme preparation, obtained from the water extract by centrifugation and subsequent filtration through ashless filter-paper (Rudra *loc cit.*), was incubated at 37° C. with 3 c.c. of phosphate buffer (pH 6·5) and 1 c.c. sugar solution (25 mgm, of glucose or mannose per c.c.); 1 c.c. of glass distilled water replaced the enzyme extract in the control. No vitamin was detected from enzyme preparations of first, second and fourth day seedlings, during 4 to 24 hours' incubation. Addtion of $Mn^{++}$ and nicotinic acid to the reaction mixture, either together or separately, had also no effect on the results. It was not possible to ascertain the effect of addition of DPN or TPN or of nicotinic acid with ATP. The presence of an aldehyde dehydrogenase was however established using the Thunberg technique.

## Changes in Glutathione

As stated earlier, this and the following studies were carried out with *mung* seeds germinated normally and in dark.

Glutathione was determined essentially by the method of Woodward and Foy.[7] An aliquot of the metaphosphoric acid + oxalic acid extract was titrated in presence of potassium iodide against 0·001N potassium iodate in oxalic acid, using starch solution as indicator. Oxidised glutathione was reduced by the addition of zinc dust. The values obtained by the two titrations were corrected for ascorbic acid. Total glutathione in germinating *mung* seeds, are given in Table VII along with the values for ascorbic acid; the oxidised (disulphide) form was very low or insignificant in all cases.

Table VII

*Changes in ascorbic acid and in glutathione in* mung *seedlings*

| | Ascorbic acid in mgm. per cent. | | | Glutathione in mgm. per cent. | | |
|---|---|---|---|---|---|---|
| Day of germination | Germinated in diffuse light | Germinated in dark | Per cent. difference | Germinated in diffuse light | Germinated in dark | Per cent. difference |
| 1 | 48·13 | 50·16 | + 4·18 | 18·14 | 18·8 | + 2·17 |
| 3 | 109·30 | 130·00 | +18·90 | 59·50 | 118·9 | +100·00 |
| 5 | 143·50 | 232·10 | +61·70 | 77·30 | 199·7 | +158·20 |

There was a steady rise in glutathione from day to day, this being more with seeds germinated in dark; such increase was paralleled by increased formation of ascorbic acid. It is significant that on the first day of germination, the increase in glutathione or in ascorbic acid is not appreciable.

### Changes in Nicotinic Acid

A dilute acid extract of the finely ground seedlings was clarified with lead acetate solution with adjustment of pH to 9·4 and centrifuged. Excess of lead was removed from solution by addition of sulphuric acid. The clear filtrate from the above was concentrated to about half the volume in presence of hydrochloric acid. To a 10 c.c. aliquot of this sloution were added 1 c.c. of 20 per cent. sodium acetate (pH 7), 2 c.c. of 2 per cent. aqueous aniline solution, followed by 6 c.c. of cyanogen bromide solution.[8] The contents were mixed and let stand for a minute. The volume was then made to 25 c.c. with the aqueous solution of aniline. The colour developed in the well mixed contents, after a further minute's standing was read off on a Klett-Summerson colorimeter using filter 54 (Table VIII). Necessary blanks were run simultaneously.

Table VIII

*Changes in nicotinic acid*

| Day of germination | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| | mgm. of nicotinic acid per cent. | | | | | | |
| Germinated in diffuse light | 1·47 | 5·27 | 5·71 | 5·93 | 15·20 | 21·57 | 21·00 |
| Germinated in dark | 1·48 | .. | 6·75 | .. | 28·80 | .. | .. |
| Per cent. difference over untreated | +0·68 | .. | +18·25 | .. | +89·48 | .. | .. |

Germination in the dark stimulated nicotinic acid synthesis in the seedling, this being pronounced on the fifth day.

### Changes in Amylase Activity

Amylase activity in aqueous extract was determined at pH 4·6 and at 40° C. by the method of Gore and Steele,[9] maltose determinations being made according to Stiles, Peterson and Fred.[10] Activity is expressed as gm. of maltose liberated in one hour by the enzyme content of 100 gm. of dry material (Table IX).

The effect of germination in dark on amylase activity was more pronounced on fifth day of germination than on the third day.

TABLE IX

*Changes in amylase activity*

| Day of germination | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| | Amylase activity (gm. of maltose per cent. | | | | | | |
| Germinated in diffuse light | 0·421 | 3·245 | 4·813 | 6·167 | 8·358 | 8·116 | 7·578 |
| Germinated in dark | 0·409 | .. | 5·658 | .. | 13·68 | .. | .. |
| Per cent. difference over untreated | −2·85 | .. | +17·56 | .. | +63·63 | .. | .. |

CHANGES IN REDUCING SUGARS

The values for reducing sugars, determined by the method of Stiles, Peterson and Fred[10] are given in Table X.

TABLE X

*Reducing sugars and effect of germination in dark*

| Day of germination | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| | gm. glucose per cent. | | | | | | |
| Germinated in diffuse light | 1·25 | 2·09 | 7·47 | 10·98 | 11·60 | 10·29 | 6·06 |
| Germinated in dark | 1·23 | .. | 8·71 | .. | 14·97 | .. | .. |
| Per cent. difference over untreated | −1·60 | .. | +16·6 | .. | +29·05 | .. | .. |

The data are in agreement with observations on amylase activity (Table IX).

CHANGES IN PHOSPHATASE ACTIVITY

Only free phosphatase was estimated, bound phosphatase being absent in germinating seeds (*cf.* [11, 12]). Activity was determined at pH 5·2 for one hour using sodium-$\beta$-glycerophosphate as substrate and is expressed as mgm. of phosphorus per 100 gm. of dry material after allowing for the free phosphate present in the glycerophosphate reagent,

TABLE XI

*Phosphatase activity of germinating seeds*

| Day of germination | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| | mgm. phosphorus per cent. | | | | | | |
| Phosphatase activity of seeds germinated in diffused light | 2·42 | 45·9 | 135·8 | 159·2 | 171·7 | 144·9 | 130·7 |
| Phosphatase activity of seeds germinated in dark | 2·45 | .. | 128·9 | .. | 196·2 | .. | .. |
| Per cent. difference over untreated | +1·33 | .. | −5·05 | .. | +14·22 | .. | .. |

## CHANGES IN INORGANIC PHOSPHATE

Phosphate values, determined celeuromolybdimetrically according to Truog and Meyer's procedure[13] and using the Klett-Summerson photocolorimeter with filter 66 in position, are given in Table XII.

TABLE XII

*Changes in inorganic phosphates*

| Day of germination | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| | mgm. phosphorus per cent. | | | | | | |
| Germinated in diffuse light | 0·378 | 2·36 | 9·70 | 10·28 | 11·49 | 9·41 | 8·17 |
| Germinated in dark | 0·380 | .. | 9·68 | .. | 13·92 | .. | .. |
| Per cent. difference over untreated | +5·29 | .. | −0·21 | .. | +20·52 | .. | .. |

As was to be expected, both phosphatase activity and free phosphate increased with germination till seedling began withering, after which they decreased. Germination in the dark resulted in increased activities on the fifth day.

## CHANGES IN DRY MATTER

The results reported here have all been expressed on dry basis. Earlier workers have in some cases given data for ascorbic acid as percentages on

fresh weight or per 100 seedlings. In Table XIII are given the dry matter content of seedlings germinated normally and in the absence of light. Values

TABLE XIII

*Changes in dry weight*

(Gm. per 100 gm. fresh seedlings)

| Day of germination | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Germinated in diffuse light | 37·60 | 36·56 | 23·34 | 12·36 | 10·94 | 9·42 | 9·41 |
| Germinated in dark | 37·19 | .. | 15·61 | .. | 5·41 | .. | .. |
| Per cent. difference over untreated | − 1·09 | .. | −33·11 | .. | −50·54 | .. | .. |

for 100 seedlings are given in Table XIV. Similar results, as influenced by cold-steeping prior to germination are shown in Table XV.

TABLE XIV

*Changes in dry matter*

(100 Seedlings)

| Day of germination | Germinated in diffuse light | | | Germinated in the dark | | | | |
|---|---|---|---|---|---|---|---|---|
| | Fresh (gm.) | Dry (gm.) | Per cent. dry matter | Fresh (gm.) | Dry (gm.) | Per cent. difference over normally germinated seeds | Per cent. dry matter | Per cent. difference over normally germinated seeds |
| | 1 | 2 | 3 | 1 | 2 | 3 | 4 | 5 |
| Ungerminated | 4·946 | 4·357 | 88·11 | .. | .. | .. | .. | .. |
| 1 | 11·635 | 3·708 | 31·85 | 11·613 | 3·701 | − 0·19 | 31· 87 | + 0·06 |
| 3 | 18·323 | 2·760 | 15·06 | 23·932 | 2·685 | − 2·72 | 11· 21 | −25·57 |
| 5 | 24·542 | 2·550 | 10·39 | 36·913 | 2·187 | −14·23 | 5·927 | −42·96 |

TABLE XV

*Effect of cold-steeping on changes in dry matter*

(100 Seedlings)

| | Untreated | | | Cold Treated | | | | |
|---|---|---|---|---|---|---|---|---|
| Day of germination | Fresh weight (gm.) | Dry weight (gm.) | Per cent. dry matter | Fresh weight (gm.) | Dry weight (gm.) | Per cent. difference over untreated | Per cent. dry matter | Per cent. difference over untreated |
| 1 | 11·635 | 3·708 | 31·85 | 8·112 | 4·157 | +12·11 | 51·23 | +60·35 |
| 3 | 18·323 | 2·760 | 15·06 | 16·384 | 3·113 | +12·79 | 19·01 | +26·23 |
| 5 | 24·542 | 2·550 | 10·39 | 23·943 | 2·849 | +11·72 | 11·90 | +14·53 |

The progressive decrease in dry matter was more when germination was in dark. In cold-treated seeds, however, this was higher as compared to untreated seedlings in the initial stages, the difference tending to decrease with the progress of germination.

## DISCUSSION

Ascorbic acid oxidase is not present on the first day of germination, and its activity is only slight on the second day. This is also true of dehydroascorbic acid. It seems therefore that the latter results only from the *in vivo* enzymic oxidation of ascorbic acid and that it does not probably act as precursor of ascorbic acid in germinating seeds. In further support of this view are the observations[4] that the proportion of ascorbic acid to dehydroascorbic acid decreases with the progress of germination and that during the initial period of germination when the cotyledons form the major portion (by weight) of the seedling, only the reduced form of the vitamin could be detected in the seedling. These results must, however, be differentiated from similar studies with maturing fruits[13,14] in which case also, the values for dehydroascorbic acid are parallel to the concomitant variations in oxidase activity[15] but with a progressive increase in the ratio of ascorbic acid to dehydroascorbic acid.

The copper-protein nature of the enzyme is generally well established but there is disagreement as to the nature of the copper in the complex, especially since ionic copper is perhaps the most powerful among the various factors causing acceleration of the auto-oxidation of ascorbic acid. Thus, Lampitt, *et al.*[16] criticised the view that the oxidase activity was exerted by

the complex and not by the ionic copper, and they, as well as, earlier, Stotz, *et al.*[17] had reported preparations whereby ionic copper could be made to possess the characteristics of the oxidase. However, it is observed here, as by others,[18,19] that the enzyme activity is many times more effective than its equivalent ionic copper action. Again, heated extract of *mung* seedlings inhibited ionic copper oxidation of the vitamin but is ineffective towards the oxidase of cucumber rind extract; in fact, extracts of first and second day seedlings enhance oxidase activity. Similar inhibition of ionic copper action on ascorbic acid by heated plant extracts has been reported by Krishnamurthy and Giri.[5] They have attributed this inhibition of copper oxidation to a 'protective factor' in the extract and have separated it from the accompanying oxidase. The unheated extracts of *mung* seedlings or of cucumber exhibits this selective inhibition of ionic copper oxidation of the vitamin, even in presence of ascorbic acid oxidase. While Stotz, *et al.*[17] consider the inactivation of the oxidase to be caused by the removal of copper from solution by precipitation or conversion to some inactive form, it is observed here that at no time during germination period could the extract of seedlings when heated together with copper solution, inactivate completely the copper action or raise the degree of inhibition exerted by the heated extract. Thus, the explanation by Stotz *et al* (*loc. cit.*) for the inactivation of the oxidase, does not appear to be tenable for the natural oxidase though it may hold true in the case of their 'synthetic' oxidase. Again, while the extracts of first and second day seedlings were able to activate the oxidase of cucumber, thus indicating the presence of the mechanism activating the oxidation of vitamin C, they inhibit completely the oxidation of ascorbic acid by ionic copper, thus suggesting the existence of an equally powerful 'protective factor'. If the ionic copper in the cucumber extract was responsible for the oxidation of ascorbic acid caused by the extract, the extract of the seedlings of first and second day should have also activated and not inhibited, the absolute ionic copper oxidation.

It seems definite therefore that the characteristics of the oxidation caused by the oxidase and by ionic copper can be differentiated. The oxidase also exhibits a partial specificity in its oxidation toward ascorbic acid and structurally related compounds and is ineffective in catalysing the oxidation of phenols, glutathione, and such other compounds.[20–24] According to Mystokowski,[25] copper oxidation is inhibited by sodium chloride, amino acids and proteins, while oxidase activity remains unaffected. Also, pyrophosphate does not exert the same amount of inhibition over oxidase as on ionic copper.[20]

The protection exerted by the 'protective factor' appears to be due to its forming a complex with copper, thereby preventing its catalytic action, rather than to its functioning as a hydrogen donator, reducing dehydroascorbic acid back to the vitamin or to its forming a compound with the vitamin stable to oxidation by ionic copper. The latter mechanisms should be equally effective towards the oxidase and ionic copper.

It is known that glutathione keeps vitamin C in its reduced form, in presence of either ionic copper or the oxidase, but it acts in different ways[26,27]; in the former case, glutathione acts by fixing copper as a non-catalytic complex, and in the latter, it acts as a hydrogen donator to dehydroascorbic acid. A chain reaction has been suugested[1,26] to explain the catalytic reduction of dehydroascorbic acid by certain plant extracts in presence of reduced glutathione. Hydrogen from plant substrates is passed on to the oxidized glutathione which in turn reduces dehydroascorbic acid formed by the oxidase action to ascorbic acid, and at the same time the reduced glutathione is reoxidized.

On the basis of the results discussed here, ascorbic acid oxidase can be considered as consisting of two parts, one which catalyses the auto-oxidation of ascorbic acid, and the other which activates the catalysis. It cannot be stated definitely whether the factor inhibiting copper oxidation and the factor accelerating the oxidase activity are identical, though both these actions are exhibited simultaneously and are thermostable. It may be recalled here that addition of manganese ions had enhanced oxidase activity of germinated *mung* extract. This mutual behaviour of the extract and manganese ions towards oxidation of ascorbic acid is striking, since it is contrary to the behaviour of the extract towards ionic copper oxidation.

$Mn^{\cdot\cdot}$ treated seeds seem to elaborate, during the initial stages of germination somewhat more ascorbic acid oxidase than untreated seeds (*cf.* [28]). A similar effect due to $Mn^{\cdot\cdot}$ is shown if it is added to the extract of seedlings. The absence of any difference in oxidase activity between untreated and $Mn^{\cdot\cdot}$ treated seedlings at later stages may, to a certain extent, be due to the decreased effective concentration of $Mn^{\cdot\cdot}$ brought about by lowering of dry matter content per unit weight of seedlings.

Pretreatment of seeds by steeping in the cold or germination in the dark results in a decrease in oxidase activity which is paralleled by increased ascorbic acid synthesis. Even though oxidase potency is more in the leaves than in the cotyledons and stems, the ratio of dehydroascorbic acid to ascorbic acid is lower in the former. This would suggest the existence in the leaves of a mechanism protecting the vitamin against enzymic oxidation, such a

mechanism being comparatively weak or non-existent in cotyledons and stems. A reducing mechanism in leaves is also inferable from the observations of Rubin, *et al.*[29] that dehydroascorbic acid, when injected in leaves, is immediately reduced to ascorbic acid.

The leaves being richer in ascorbic acid and its oxidase, as compared to the cotyledons and the stems, may mean that these are formed in the leaves and then distributed to the other parts. It is also possible that the unknown precusor to ascorbic acid in the cotyledons and stems are supplied to the leaves, they themselves lacking the capacity to bring about this transformation.

Pett has reported that glutathione and ascorbic acid appear at the same time and that they both increase in amount during sprouting.[30] Of especial interest is the observed agreement between the increase in glutathione content of the seedlings and the proportionate stimulation in ascorbic acid formation in seeds germinated in dark. Hopkins[26] and, later, others have shown that the oxidized form of vitamin C is reduced to its original form in presence of a large excess of reduced glutathione. The enzyme concerned, dehydroascorbic acid reductase, has been isolated,[1,2] although Schultze, *et al.*[31] could obtain no evidence of the reduction being enzymic in character. The necessity for a large excess of glutathione for reduction of dehydroascorbic acid to ascorbic acid has been emphasised by all workers. Since seeds germinated in dark have higher ascorbic acid and glutathione, with less dehydroascorbic acid and reduced oxidase activity it would seem justifiable to assume the presence of an interlinked mechanism similar to the one recorded by earlier workers. However, the effect of germination in dark cannot be explained on the basis of the effect of glutathione on dehydroascorbic acid alone, on account of the difference in the total ascorbic acid contents of seeds germinated in dark and those germinated under normal conditions. Obviously therefore there are other factors which could oxidise ascorbic acid to both reversible and irreversible forms and which are comparatively more active under normal conditions of germination.

Irradiation hastens the oxidative destruction of ascorbic acid.[4] Hence, in seeds germinated in dark, not only is there a check on enzymic oxidation through the greater formation of glutathione but photo-oxidation is minimised.

The pronounced ascrobic acid synthesis in embryos grown on nutrient media containing sugars such as glucose and mannose[4,32,33] would substantiate the view that these sugars are precursors in the biosynthesis of ascorbic acid.[34–38] The increase in reducing sugars resulting from germina-

tion in the dark should therefore be expected to stimulate the vitamin formation. The observation of Smith, *et al.*[39] that, in the diurnal variations in ascorbic acid of growing plants, the vitamin is present at its highest level usually during mid-day, may also be explained on the basis of this hypothesis. For, at this period, the photo-synthetic activities and hence the formation of sugars is the maximum. In growing plants, sugars, being products of photosynthesis, will be formed in proportion to the photo-energy received, whereas in germination, these result from amylolysis of reserve carbohydrates. The influence of chlorophyll on ascorbic acid formation appears therefore to be indirect and applicable only to the growing plant.

The role of pyridino-protein enzymes and of phosphates in dehydrogenation mechanisms is well known and, doubtless, the pronounced increase in nicotinic acid, the enhanced amylolysis resulting in increased amounts of reducing sugars, and the stimulation of ascorbic acid formation, all are closely interlinked. However, attempts to synthesise ascorbic acid from sugars through the dehydrogenases of seedling extracts, in presence of $Mn^{++}$, the supposed co-enzyme[6] or nicotinic acid were not successful. These results do not preclude the possibility that ascorbic acid, while not being a product of direct dehydrogenation of sugars, is formed through some complex mechanism from metabolized sugars.

Seed germination is accompanied by increased absorption of water and hydrolysis of the reserve food in the cotyledons into simple, easily assimilable substances. This process of water absorption continues till all the stored food material is exhausted when, due to the lack of food—unless provided from without—the seedling begins withering. Thus, the moisture content of seedlings reaches a maximum value and forms, in a sense, a measure of the extent of hydrolysis within the seedling. The increased moisture content of seeds germinated in the dark would therefore indicate activation of metabolic changes.

The data on dry matter of 100 seedlings show that this decreases with germination and is more with seeds germinated in the dark; thus, on the fifth day of germination the dry matter in a seedling is about 15 per cent. less than in a normally germinated seed. In asserting the effect of any treatment, it would seem necessary that the activities of equal number of seedlings germinated under different conditions should be the basis of comparison. Even when calculated on this basis, seeds germinated in dark show enhanced activities. Thus, for example, from Table VIII, ascorbic acid contents per 100 seedlings, germinated under normal conditions, on third and fifth day are 3·016 and 3·659 mgm. while the corresponding values for germination

in dark are 3·489 and 5·077 mgm. respectively; the increased synthesis in the latter case is 15·68 per cent. on the third and 38·31 per cent. on the fifth day.

The progressive degradation of the stored food material in the seeds during germination would obviously include the change responsible for the formation of the precursor for ascorbic acid. It does not always follow, however, that the formation of the vitamin should be related to the rate of hydrolysis of the reserve plant material or in other words to the dry matter content; all that can be stated is that vitamin C synthesis is related to one of the many hydrolytic changes undergone by the seed material and that this particular hydrolysis may not be indicated in its extent by the dry matter content. That this is so can be seen from the fact that cold-treated seeds elaborate more vitamin C than untreated seeds although there is no corresponding greater dissolution of the seed material.

## Summary

In continuation of observations on the changes in ascorbic acid, dehydroascorbic acid and total ascorbic acid during germination of seeds, the day to day changes in the activities of ascorbic acid oxidase, glutathione, reducing sugars, free phosphates, nicotinic acid, amylase and phosphatase have been followed.

Germination in the dark has generally tended to enhance the various activities, except that of ascorbic acid oxidase. The probable bearing of these observations on the mechanism of ascorbic acid formation, has been discussed.

That enzymic and ionic copper oxidation of vitamin C are distinct is shown from studies with heated extracts of germinated *mung*.

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## References

1. Crook .. *Biochem. Jour.*, 1941, **35**, 226.
2. ——— and Morgan .. *Ibid.*, 1944, **38**, 10.
3. Sreenivasan and Wandrekar .. *Current Science* (In Press).
4. ——— ——— .. *Proc. Ind. Acad. Sci.*, 1950, **32**, 143.
5. Krishnamurthy and Giri .. *Jour. Ind. Chem. Soc.*, 1941, **18**, 7, 191. *Indian Jour. Med. Res.*, 1941, **29**, 71.
6. Rudra .. *Nature*, 1943, **151**, 641.
7. Woodward and Foy .. *Jour. Biol. Chem.*, 1932, **97**, 465.
8. Swaminathan .. *Indian Jour. Med, Res.*, 1940, **27**, 677.

9. Gore and Steele .. *Ind. Eng. Chem.* (*Anal. Ed.*), 1935, **7**, 324.
10. Stiles, Peterson and Fred .. *Jour. Bact.*, 1926, **12**, 427.
11. Sarma and Giri .. *Ann. Biochem. & Expt. Med.*, 1942, **2**, 17.
12. Booth .. *Biochem. Jour.*, 1944, **38**, 355.
13. Zilva, Kidd and West .. *New Phytol.*, 1938, **37**, 345.
14. West and Zilva .. *Biochem. Jour.*, 1944, **38**, 105.
15. Rangnekar .. *Proc. 33rd Ind. Sci. Congr.*, Part III Abstracts, 1946, **41**.
16. Lampitt, Clayson and Barnes .. *Biochem. Jour.*, 1945, **39**, XV, XVI.
17. Stotz, Harrer and King .. *Jour. Biol. Chem.*, 1937, **119**, 511.
18. Banga and Szent-Györgyi .. *Z. physiol. Chem.* 1938, **225**, 57.
19. Lovett-Janison and Nelson .. *Jour. Amer. Chem. Soc.*, 1940, **62**, 1409.
20. Tauber and Kleiner .. *Proc. Soc. Expt. Biol. & Med.*, 1935, **32**, 577.
21. Zilva .. *Biochem. Jour.*, 1936, **30**, 1215.
22. Johnson and Zilva .. *Ibid.*, 1937, **31**, 438.
23. Rosenberg .. *Skand. Arch. Physiol*, 1937, **76**, 119.
24. Snow and Zilva .. *Biochem. Jour.*, 1938, **32**, 1926.
25. Mystkowski .. *Ibid.*, 1942, **36**, 494.
26. Hopkins and Morgan .. *Ibid.*, 1936, **30**, 1446.
27. Barron, Barron and Klemperer *Jour. Biol. Chem.*, 1936, **116**, 563.
28. Shen, Hsieh and Chen .. *Biochem. Jour.*, 1945, **39**, 107.
29. Rubin, Syssakian and Lutikova *C. R. Acad. U. R. S. S.*, 1937, **15**, 495.
30. Pett .. *Biochem. Jour.*, 1936, **30**, 1228.
31. Schultze, Stotz and King .. *Jour. Biol. Chem.*, 1938, **112**, 395.
32. Ray .. *Biochem. Jour.*, 1934, **28**, 996.
33. Ahmed, Quereshi, Babbar and Sawhney .. *Ann. Biochem. & Expt. Med.*, 1946, **6**, 29.
34. Fukumoto and Shimomura .. *Jour. Agri. Chem. Soc.* (*Japan*), 1937, **13**, 613.
35. Siegal and King .. *Jour. Biol. Chem.*, 1936, **116**, 489.
36. ——— —— .. *Jour. Pharmacol. & Expt. Therap.*, 1937, **59**, 468.
37. Smythe and King .. *Jour. Biol. Chem.*, 1942, **142**, 529.
38. Roy, Roy and Guha .. *Nature*, 1946, **158**, 238.
39. Smith and Gillies .. *Biochem. Jour.*, 1940, **34**, 1312.

# STUDIES IN CROP PHYSIOLOGY

## Interrelation between Leaf Area, Leaf Indices and Drought Resistance in Sugarcane

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### Introduction

In a previous communication,[20] an attempt was made to elucidate the relation between certain internal and external factors of growth and expansion of leaf surface in sugarcane. Relative effects of tillers, leaf age, plant development and environment showed that pre-monsoon period was more suited for better development of leaf surface in all varieties. Tillers developed during hot and dry season invariably showed better development of leaf surface even during later periods of life-cycle. Drought-resistant forms, for instance, Rheora showed lowest value for different leaf indices. If low leaf index is at all associated with drought resistance other varieties, *e.g.*, Co 205, Co 313, Co 356 and Cos 76 approach in their leaf characteristics a behaviour more or less similar to that of drought-resistant Rheora.

In general, leaf indices have been utilized for taxonomic and agronomic investigations and for determining physiological responses to different conditions of growth in nutritional studies.[39] In *Achras zapota* leaves, significant differences in blade length were noticeable among four local populations.[8] Even the individual blades in *Phaseolus vulgaris* showed most diverse kinds of growth.[28] Positive correlation between leaf area and fresh weight,[26, 30, 32] between leaf surface and width of widest foliage leaf,[19] between leaf area and dry matter residue, and between leaf area, and length and width of leaf has been noted by many investigators[7, 16] though observations on the contrary, are also on record.[33] Root production and leaf area on the cutting were interrelated.[3] Surface tension of the sap of leaf lamina decreased as the area increased.[14] Similarly,

yield under field conditions was mainly determined by leaf area.[38] In rice, marked effect on yield and quality was observed by reducing leaf area.[34] Sugarcane varieties differed significantly in their dry matter output in relation to mean leaf area.[18] In deciduous fruits, species with narrow leaves have been considered drought-resistant and *vice versa*.[17] Comparatively, Sorghum was more drought-resistant than corn because in addition to other features, the former was less leafy.[22] In view of its important role in growth of plants, rapid methods of estimating leaf area, by multiplying linear measurements with a leaf factor,[7] or by mathematical calculations have been evolved.[1, 4, 16, 27] Use of photoelectric equipment, chartometers, planimeters and photosensitised papers have also been recommended.[2, 6, 9, 10, 11, 12, 13, 15, 21, 31, 35, 36, 37, 40]

The present paper produces further evidence on the interrelation between leaf indices and leaf surface in sugarcane. It attempts to elucidate—(i) the extent to which expansion in leaf area is related with length, breadth, length ×breadth, dry weight and water content of leaves; (ii) the correlation, if any, between these physiological attributes and leaf area; (iii) the practical utility of linear measurements in determination of total leaf surface; and (iv) growth and leaf characteristics of different sugarcane varieties in relation to the major problem of drought resistance.

## Experimentation

These investigations were conducted on six sugarcane varieties, *viz.*, Co 290, Co 453, Co 312, Co 385, Co 205 and Rheora grown on the Experimental Farm of the College, during the cropping season of 1946-47. Towards the end of the adolescent period (270 days), one healthy stool of these varieties was sampled. Different tillers in the stool were arranged in order of increasing shoot length and leaf area exposed by the green foliage on individual shoots was determined separately. Measurements of other leaf characters such as length, breadth, dry weight and per cent. moisture content were made on these leaves as indicated in the previous paper.[20]

To assess the nature of relation between different leaf indices and area exposed by the foliage, correlation graphs were drawn. Various pairs of comparison such as leaf area *versus* (i) leaf length, (ii) leaf width, (iii) product of length and breadth, (iv) dry weight, and (v) per cent. moisture content were illustrated by these figures. Correlation coefficients between these pairs of characters in the aforesaid six varieties were also calculated and their standard error determined by the formula,[29]

$$r = \frac{d_x \cdot d_y}{\sqrt{d_1^2 \cdot d_2^2}} \quad (1)$$

$$\text{S.E.}_r = \frac{\sqrt{1 - r^2}}{\sqrt{n - 2}} \quad (2)$$

where,

$r$ = correlation coefficient; $d_x.d_y$ = product deviation; $d_1^2$ and $d_2^2$ are deviation squares from means; S.E.$_r$ is standard error of correlation coefficient and $n$ the number of plant.

Correlation coefficients being known, attempts were made to trace specific relation, if any, between length and breadth measurements and area exposed by leaves. Observations showed that the ratio $\frac{\text{Actual leaf area}}{\text{Leaf length} \times \text{leaf breadth}}$ in twenty-five leaves of each of the six varieties was more or less constant (Table I). The average value for this constant K was found to

TABLE I

*Actual leaf area/length× breadth ratio in 25 leaves of six sugarcane varieties*

| Leaf No. | Rheora | Co 205 | Co 312 | Co 385 | Co 290 | Co 453 |
|---|---|---|---|---|---|---|
| 1 | 0·649 | 0·627 | 0·523 | 0·600 | 0·734 | 0·724 |
| 2 | 0·636 | 0·617 | 0·668 | 0·670 | 0·692 | 0·709 |
| 3 | 0·794 | 0·653 | 0·691 | 0·702 | 0·649 | 0·761 |
| 4 | 0·697 | 0·626 | 0·691 | 0·730 | 0·712 | 0·765 |
| 5 | 0·751 | 0·758 | 0·710 | 0·726 | 0·745 | 0·889 |
| 6 | 0·705 | 0·658 | 0·637 | 0·614 | 0·637 | 0·743 |
| 7 | 0·651 | 0·679 | 0·616 | 0·701 | 0·761 | 0·738 |
| 8 | 0·621 | 0·685 | 0·675 | 0·734 | 0·760 | 0·633 |
| 9 | 0·719 | 0·623 | 0·637 | 0·751 | 0·729 | 0·646 |
| 10 | 0·752 | 0·591 | 0·695 | 0·734 | 0·718 | 0·699 |
| 11 | 0·530 | 0·659 | 0·561 | 0·740 | 0·822 | 0·481 |
| 12 | 0·673 | 0·580 | 0·641 | 0·730 | 0·775 | 0·684 |
| 13 | 0·673 | 0·642 | 0·651 | 0·893 | 0·813 | 0·619 |
| 14 | 0·789 | 0·697 | 0·711 | 0·748 | 0·741 | 0·647 |
| 15 | 0·701 | 0·652 | 0·680 | 0·781 | 0·633 | 0·689 |
| 16 | 0·640 | 0·753 | 0·634 | 0·706 | 0·712 | 0·678 |
| 17 | 0·664 | 0·647 | 0·640 | 0·754 | 0·622 | 0·843 |
| 18 | 0·675 | 0·663 | 0·788 | 0·720 | 0·708 | 0·870 |
| 19 | 0·667 | 0·654 | 0·715 | 0·693 | 0·777 | 0·847 |
| 20 | 0·581 | 0·778 | 0·846 | 0·697 | 0·798 | 0·764 |
| 21 | 0·549 | 0·661 | 0·820 | 0·600 | 0·605 | 0·887 |
| 22 | 0·627 | 0·636 | 0·890 | 0·651 | 0·679 | 0·623 |
| 23 | 0·635 | 0·617 | 0·853 | 0·603 | 0·728 | 0·707 |
| 24 | 0·651 | 0·609 | 0·872 | 0·843 | 0·728 | 0·857 |
| 25 | 0·797 | 0·718 | 0·872 | 0·807 | 0·686 | 0·750 |
| Total .. | 16·827 | 16·493 | 17·717 | 17·928 | 17·974 | 18·263 |
| Mean K.. | 0·673 | 0·659 | 0·708 | 0·717 | 0·718 | 0·730 |

vary slightly with the variety, for instance, in case of Rheora and Co 205 (varieties with relatively low leaf indices) it was lowest (0·659 — 0·673). In the group with medium leaf indices, *e.g.*, Co 312 and Co 385, this constant showed a value of 0·708—0·717. In Co 290 and Co 453, K varied between 0·718—0·730. Varieties with high leaf indices in a way tended to show a higher value of this constant K. This important relationship between linear measurements and leaf area is employed in determination of total leaf surface. How this has been helpful is illustrated in the following pages.

Finally, number of shoots per stool, number of green leaves, total leaf surface* and average leaf area were determined in these six varieties to elucidate their growth characteristics.

## Experimental Results

### A. *Relation between Leaf Area and Other Leaf Characteristics in Sugarcane*

Previous studies on nature of transpiring surface,[20] showed that the varieties investigated could be conveniently classified into three categories: (I) those with low values for leaf character, *e.g.*, Rheora, Co 205, Cos 76, Co 356, and Co 313; (II) those with intermediate values, *e.g.*, Co 312, Co 213, Co 385, Co 421 and Co 299 and (III) those with high values, *e.g.*, Co 290 and Co 453. It has now been attempted to further trace the relation between leaf area and other leaf attributes in two representative varieties belonging to each of these three classes. Rheora and Co 205, representatives of class I, Co 312 and Co 385 of Class II and Co 290 and Co 453 as representatives of Class III were selected for these studies.

When log area was plotted against log leaf length in all the six varieties, correlation graphs were typical; all the points arranged in an ascending straight line showing thereby that area increased in direct proportion to the length of blade (Fig. 1). The greater the leaf length, the larger was the leaf area exposed by the blade. This relationship was significantly noted in all varieties.

Relation between width of leaf and area was not so characteristic (Fig. 2). In varieties of Class I (Co 205 and Rheora) relationship was more linear than

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* Total leaf surface = Area of both the surface, *i.e.*, twice the area determined by planimeter.

in varieties belonging to Classes II (Co 312 and Co 385), or III (Co 290 and Co 453). While points in Rheora and Co 205 were more closely distributed along a certain direction, those in case of the other four varieties were less characteristically distributed. Nevertheless, the general behaviour did indicate that width of leaf was positively correlated with leaf surface.

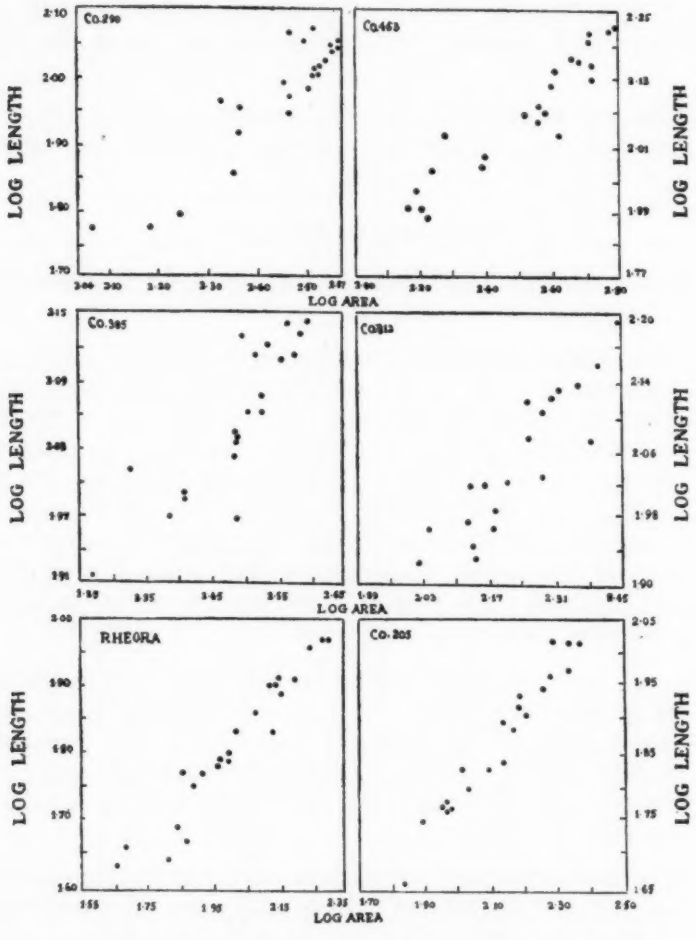


FIG. 1. Relation between leaf length and leaf area of sugarcane varieties

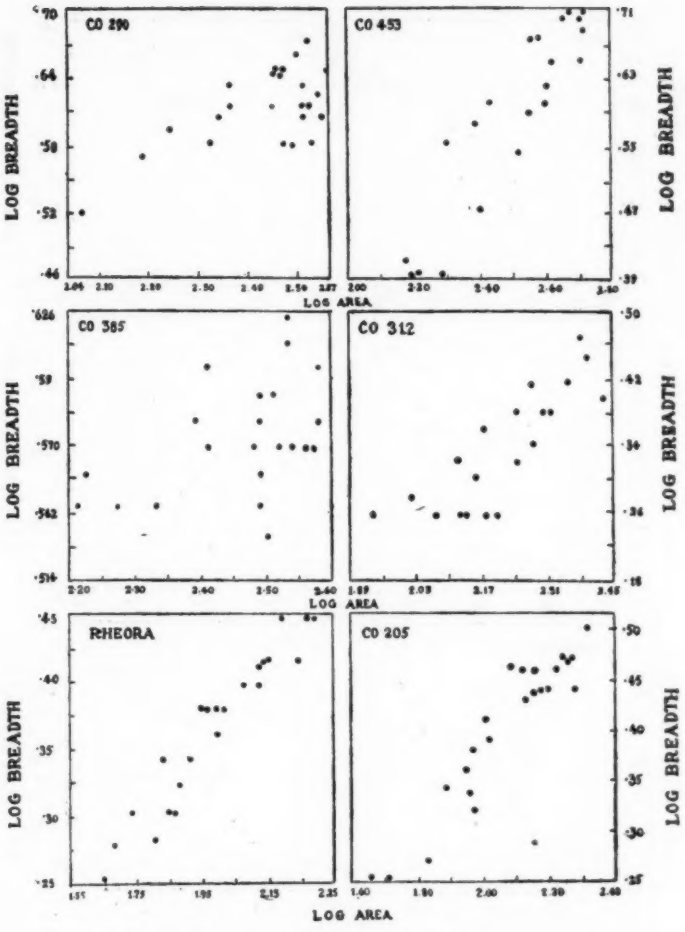


FIG. 2. Relation between leaf area and leaf width of sugarcane varieties

The product of length and width showed once again a clear positive correlation with leaf area in all the six varieties (Fig. 3). In this relationship, the higher the product, the larger was the leaf area in all the varieties.

On plotting dry weight of leaf against leaf area, it was observed that this character also followed a relation similar to that of either length *versus*

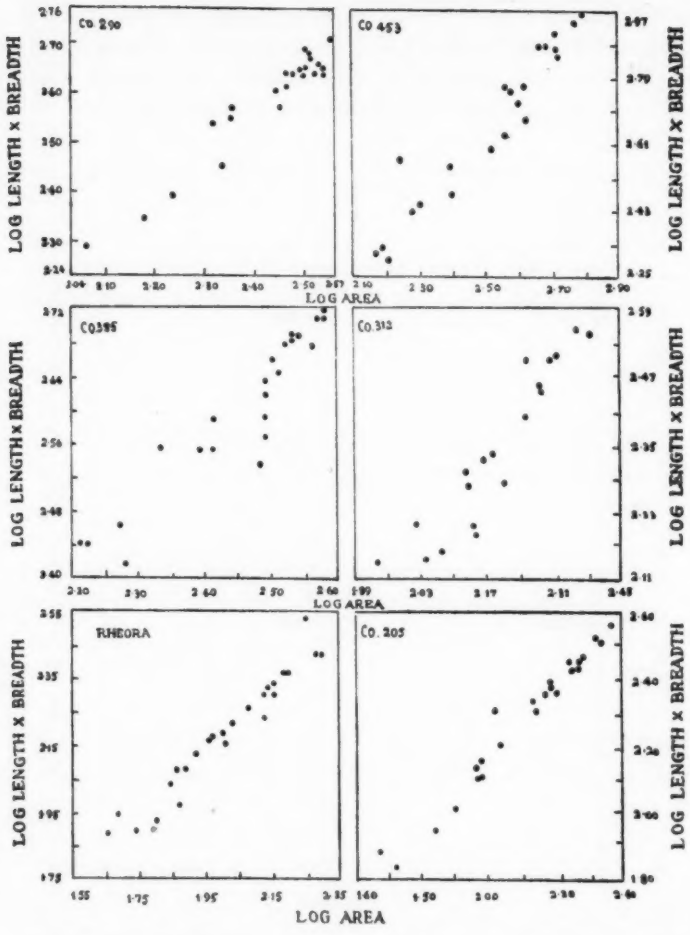


FIG. 3. Relation between leaf area and Log Length × breadth in different sugarcane varieties

area, or product of length and breadth *versus* area. The larger the area, the greater was the dry weight of individual leaves (Fig. 4).

Leaf area and moisture content did not appear to be related as significantly as the above characters except in Co 290 and Co 453 where an increase in water content showed tendencies to decrease leaf area. The

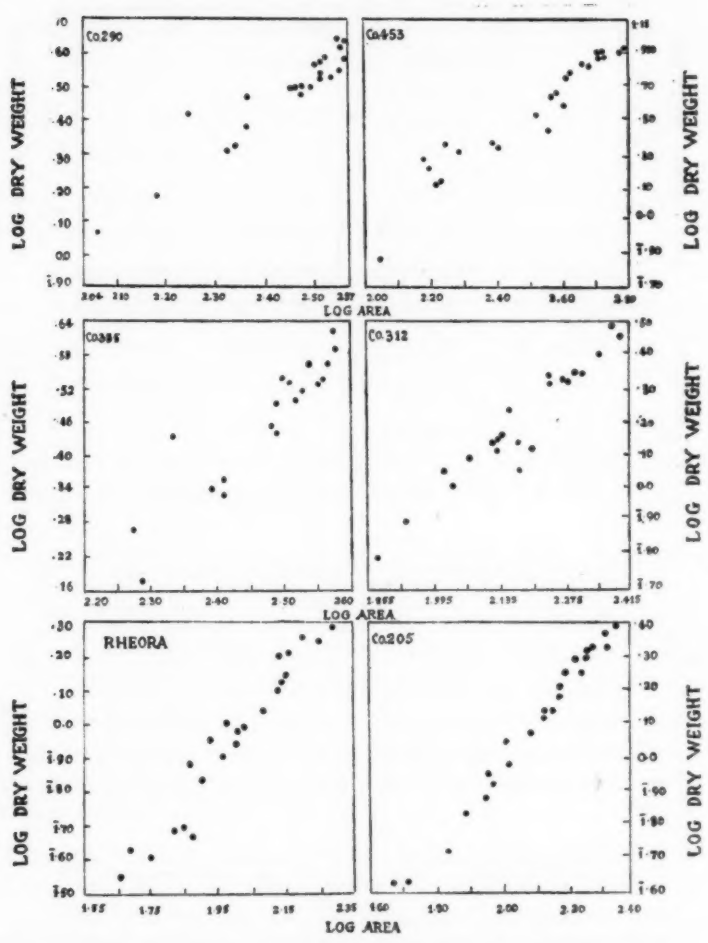


FIG 4. Relation between leaf area and dry weight in different sugarcane varieties

relation between these two characters in other forms, *e.g.*, Co 385, Co 312, Co 205 and Rheora was less evident. This was clear from Fig. 5 where the correlation graphs in the four varieties stated above showed indiscriminate distribution of points as against more systematic distribution in Co 290 and Co 453.

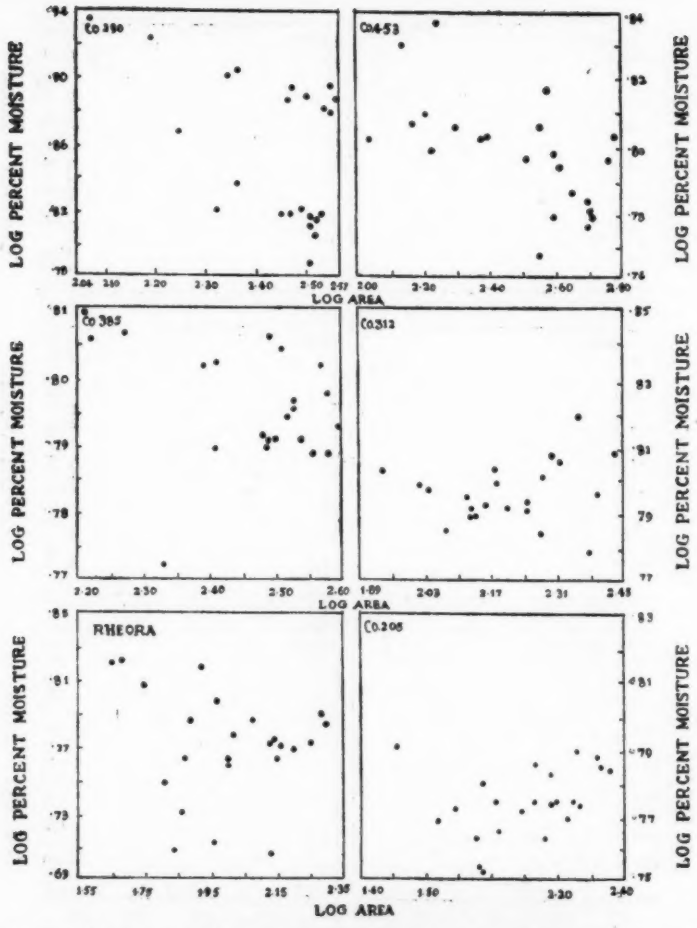


FIG 5. Relation between leaf area and moisture content in different sugarcane varieties

B. *Correlation Coefficients between Leaf Area and Other Leaf Attributes*

To further evaluate the extent of above relationship, correlation coefficients were calculated between (i) leaf area and leaf length, (ii) leaf area and width of leaf, (iii) leaf area and dry weight, (iv) leaf area and per cent. moisture content and (v) leaf area and product of leaf length and leaf breadth. These were determined in all six varieties representative of the hree classes indicated above (Table II).

TABLE II

*Correlation coefficient between leaf area and other leaf characters in six varieties of sugarcane*

| Variety | Area × Length | Area × Breadth | Area × Dry Wt. | Area × % W. C. | Area (L × B) |
|---|---|---|---|---|---|
| Rheora .. | 0·97±0·050 | 0·92±0·081 | 0·98±0·039 | −0·108±0·204 | 0·95±0·064 |
| Co 205 .. | 0·97±0·050 | 0·97±0·050 | 0·99±0·020 | 0·002±0·206 | 0·99±0·020 |
| Co 312 .. | 0·91±0·085 | 0·87±0·100 | 0·95±0·064 | −0·074±0·206 | 0·94±0·068 |
| Co 385 .. | 0·93±0·075 | 0·67±0·156 | 0·96±0·058 | −0·32 ±0·195 | 0·95±0·064 |
| Co 290 .. | 0·92±0·081 | 0·72±0·143 | 0·95±0·064 | −0·507±0·154 | 0·95±0·064 |
| Co 453 .. | 0·95±0·064 | 0·91±0·085 | 0·96±0·058 | −0· 62±0·162 | 0·96±0·058 |

Leaf area and leaf length showed very high and significant correlation. In all varieties, coefficients ranged between $0·91 \pm 0·085$ to $0·97 \pm 0·050$ (Table II).

In case of leaf width the range of variation was wider. Co 385 exhibited a coefficient of $0·67 \pm 0·156$ while Co 205 indicated a value of $0·97 \pm 0·050$. Within these were located the correlation coefficient of other varieties. In general, all varieties showed high correlation coefficient between these characters; Rheora and Co 205 exhibited relatively higher value than others.

Correlation coefficient between leaf area and dry weight again exhibited very high and significant values irrespective of the classes to which the varieties belonged; the coefficient varied slightly between the range $0·95 \pm 0·064$ to $0·99 \pm 0·020$.

Per cent. moisture content showed no significant correlation in varieties of Class I or II. In Co 290 and Co 453 significantly negative correlation was recorded between area and per cent. moisture content. The larger the leaf area the lower was the moisture content of the leaf.

Leaf area and length × breadth relationship exhibited very high and significantly positive correlation. The range differed slightly from $0·94 \pm 0·068$ to $0·99 \pm 0·020$. All varieties behaved identically.

C. *Utility of Linear Measurements in Estimation of Leaf Area*

Preliminary observation on the ratio, $\frac{\text{Actual leaf area}}{\text{leaf length} \times \text{breadth}}$ showed that in all the six varieties more or less similar values were obtained (Table I).

The average value of this ratio K differed slightly among varieties. It was lowest in Rheora and Co 205—varieties with low leaf indices, intermediate in case of Co 312 and Co 385—varieties with medium values of leaf attributes and relatively high in Co 290 and Co 453—varieties with high leaf indices. The entire range varied between 0·659 to 0·730.

This factor K appears therefore, to be an important physiological constant which may effectively be utilised for estimation of leaf area on the basis of simple linear measurements of leaf. This relation may be mathematically expressed as follows:—

$$A = (L \times B)\,K, \tag{1}$$

where A = leaf area, L = leaf length, B = leaf width at the widest region, and K = leaf factor for the variety. This relationship may, however, be expressed in another way:—

$$\text{Log } A = \text{Log } L + \text{Log } B + \text{Log } K. \tag{2}$$

If the number of leaves is large, this expression may slightly be modified to give $\Sigma A$ or the total leaf area exposed on the plant on the basis of $\Sigma$ leaf length and $\Sigma$ leaf width of the total population of leaves (N). In this case equations (1) and (2) are modified as follows:

$$\Sigma A = \frac{(\Sigma L \times \Sigma B)}{N}.K \tag{3}$$

$$\text{or } \text{Log } \Sigma A = \text{Log } \Sigma L + \text{Log } \Sigma B + \text{Log } K - \text{Log } N \tag{4}$$

Equations (2) and (4) therefore express the relation between linear measurements and leaf area in a variety. Utility of these formulæ in actual practice has been found by determining the percentage difference between the area determined by the planimeter and that calculated by the formula (4). Percentage difference from actual leaf area is sufficiently low with an average difference of 2·44 per cent. for six varieties (Table III).

This method of estimating area has certain advantages. First, it does not involve the use of any costly appliance. Secondly, it helps in determining leaf area on a growing plant without detaching the leaves. Thirdly, it enables the leaf area to be estimated well within 10 minutes of the observation. All that is needed is to have the following determinations: (i) Determine the value of K from a population of leaves numbering 25-30 on the crop; (ii) Calculate the total length and total width of all the leaves on the plant; and (iii) knowing the values of K, $\Sigma L$ and $\Sigma B$ apply formula (4) and calculate the total area on the plant. Value of K once determined may be helpful for the entire life-cycle.

TABLE III

*Relation between linear measurements and area in different sugarcane varieties*

| Variety | Total length cm. ΣL | Total width cm. ΣB | Product Σ(L×B) | Actual area sq. cm. | K | Calculated area (Formula 4) | Per cent. Difference |
|---|---|---|---|---|---|---|---|
| Rheora .. | 1671·0 | 58·9 | 4137·2 | 2793·0 | 0·673 | 2650·0 | 5·11 |
| Co 205 .. | 1897·0 | 66·3 | 5236·2 | 3412·5 | 0·659 | 3316·0 | 2·82 |
| Co 312 .. | 2662·0 | 52·3 | 5781·1 | 3986·7 | 0·708 | 3943·0 | 1·09 |
| Co 385 .. | 2723·0 | 93·0 | 10171·2 | 7348·6 | 0·717 | 7264·0 | 1·15 |
| Co 290 .. | 2376·0 | 101·0 | 9801·2 | 7067·6 | 0·718 | 6892·0 | 1·07 |
| Co 453 .. | 2870·0 | 99·8 | 12156·3 | 8665·2 | 0·730 | 8364·0 | 3·47 |
| | | | Mean .. | | 0·700 | .. | 2·44 |

D. *Growth and Leaf Characteristics of Different Sugarcane Varieties*

Preliminary observations on number of tillers, total number of green leaves, total leaf surface and average leaf surface in six varieties further indicate some specific relation between the general behaviour of these varieties and their leaf measurements (Table IV). For instance, Rheora and Co 205 which represent types with low values of leaf attributes were characterised in their growth performance by large number of tillers, greater leaf number but very low total and average leaf surface. On the other hand, Co 453 and Co 290 which belong to the other class of high leaf attributes

TABLE IV

*Growth characteristics and leaf area expansion in six varieties of sugarcane*

| Variety | Tiller number | Total No. green leaves | Total leaf surface exposed sq. cm. | Av. leaf area sq. cm. |
|---|---|---|---|---|
| Co 453 .. | 6 | 31 | 20566·7 | 663·43 |
| Co 290 .. | 6 | 34 | 17530·40 | 515·60 |
| Co 385 .. | 6 | 35 | 17914·40 | 511·84 |
| Co 312 .. | 6 | 30 | 10147·40 | 338·24 |
| Co 205 .. | 9 | 46 | 13070·00 | 284·13 |
| Rheora .. | 8 | 40 | 8578·00 | 214·45 |

showed maximum leaf surface and highest average leaf surface. The remaining two varieties occupied an intermediate position. Rheora exhibited poor total leaf surface and low average leaf area as compared to Co 453, Co 290, Co 385, and Co 312. If poor leaf surface and low average leaf area represented the drought-resistant characteristics of Rheora, Co 205 approached Rheora most in its growth behaviour.

## Discussion

A critical survey of the above observations indicates that Rheora is drought resistant mainly because it exposes poor leaf surface,[22] with leaves of smaller size than other varieties. Co 205 approaches this drought-resistant variety most in so far as its leafy growth is concerned. Length of the leaf shows considerable reduction, though width variations are relatively less in Rheora. Number of tillers, on the contrary, are usually high in this variety.[23] How far suppression of leaf surface expansion on one hand and increased tillering on the other are related to inhibition of apical growth of main stem and consequent development of lateral buds situated towards the base in absence of apical dominance, is difficult to be specifically ascertained. To what extent such a behaviour is related to acquired habit of low form and bushy growth is equally unknown in view of meagre information on drought resistance in sugarcane varieties. Comparative studies in sweet corn, however, point out that the date when tillers attach themselves to main shoot seem to be of greater significance than the number of tillers in determining drought resistance.[24]

Irrespective of the intrinsic behaviour of different varieties, certain facts regarding physiology of leaf growth are evident from these experimental results. While maximum expansion of leaf surface is a varietal characteristic, development of leaf surface appears largely to be determined by certain other factors, for instance, the linear growth of surface cells in these varieties. This specific relation between linear growth and leaf area is fully illustrated by data recorded in Table I. Leaf area is invariably found to be proportional to the product of leaf length and breadth; a leaf factor of 0·659—0·730 expresses this relationship between the two characters. It is incidentally noted that in Rheora and Co 205 this leaf factor is slightly lower than in other varieties. What specific relationship exists between this low value of the constant and capacity to withstand drought is too premature to indicate. All that can be visualized is that leaf factor, leaf surface expansion and drought resistance appear interrelated.

Aside from linear characteristics of leaves, water content shows some correlation with leaf area. At least in two varieties Co 290 and Co 453

a significant negative correlation between the two, points out the possibility that leaves with low water content tend to produce larger area (Table II). No consistent relation between moisture content and leaf area and moisture content and drought resistance is evident even in Rheora. It may, however, be pointed out that in varieties with high leaf attributes such as Co 290 and Co 453 association of greater leaf area with low water content is significant. This indicates some specific effect of water content upon the general physiology of leaf growth and more particularly upon the development of number and size of epidermal cells which largely determine area.[25] Direct evidences in these directions are, however, lacking.

Leaf surface in sugarcane is largely limited by dry weight of the leaf material. High positive correlation between leaf area and dry weight in all varieties indicates that a certain mass of living and non-living tissues is a prerequisite to expansion of leaf surface. This is natural in view of the fact that continued expansion is possible only under conditions when growth and development of individual leaf cell progresses uninterrupted by deficient supplies of protoplasm and other requisite material substratum. If dry matter is assumed to represent both living and non-living material, unhampered growth of individual cells would be possible only when increase in dry weight keeps pace with leaf surface expansion. This is invariably true of all the varieties. Along with linear measurements of length and width, dry weight of individual leaves, therefore, is an equally important factor determining leaf surface expansion. How these different characteristics of leaves are interrelated with leaf area is amply explained by the results recorded above. Water content appears less important. The mechanism of response of different varieties to the condition of growth, however, is difficult to be explained in its entirety only on the basis of these leaf characteristics of different varieties. Of equal importance are the major problems of water relations and growth performance in relation to nutrient status. Further light in these directions would be thrown in subsequent communications.

## Summary

This paper elucidates the interrelation between leaf area and other leaf characteristics in six varieties of sugarcane grown under field conditions. Relation between these features and drought resistance in sugarcane is also analysed.

Drought-resistant behaviour of Rheora is associated with poor leaf surface and small size of leaves. Co 205 approaches this variety most in the characteristics of its leafy growth.

Leaf surface expansion is found to be determined by three conditions, namely, increase in (i) leaf length, (ii) leaf width and (iii) dry weight of leaves. High positive correlation between these factors and leaf area indicates that these characteristics are highly correlated. The possibilities of water content showing negative correlation with leaf area is also pointed out.

Relation between leaf area and linear measurements of length and breadth is expressed by the following two equations:

For a single leaf:—

$$\text{Log } A = \text{Log } L + \text{Log } B + \text{Log } K \qquad (1)$$

For a population of leaves:—

$$\text{Log } \Sigma A = \text{Log } \Sigma L + \text{Log } \Sigma B + \text{Log } K - \text{Log } N, \qquad (2)$$

where

A is leaf area, L = leaf length, B is leaf breadth, K is leaf factor and N is number of leaves.

The leaf factor K appears to be an important physiological constant representing the relation between linear growth (L × B) and leaf area. Its relatively low value in drought-resistant Rheora and also in Co 205, as against its relatively high value in other varieties with high leaf indices—Co 290, Co 453, Co 385 and Co 312—is characteristic.

## References


1. Asano, M. .. "Some contrivances for ready determination of leaf area in pear trees," *Jour. Hort. Assoc. Japan*, 1934, **5** (1), 133–38.
2. Baines, R. C. and Benedict, H. M. .. "Rate of increase in area of apple—sugar leaves," *Phytopath.*, 1935, **25** (3), 373–75.
3. Calma, V. C. and Richey, H. W. .. "Influence of amount of foliage on rooting of Coleus cuttings," *Proc. Amer. Soc. Hort. Sci.*, 1930 (31), **27**, 457–62.
4. Caruthers, R. S. .. "A scale for measuring areas of Ribes leaves," *Phytopath.*, 1929, **19** (4), 399–405.
5. Chandler, W. H. .. "The dry matter residue of trees and their products in proportion to leaf area," *Proc. Amer. Soc. Hort. Sci.*, 1934, **31**, 39–56.
6. Darrow, G. M. .. "Method of measuring strawberry leaf areas," *Pl. Physiol.*, 1932, **7** (4), 745–47.
7. Davis, J. F. .. "The relationship between leaf area and yield of the field bean with a statistical study of methods for determining leaf area," *Jour. Amer. Soc. Agron.*, 1940, **32** (5) 323–29.
8. Eagler, F. E. .. "Some statistics of *Achras zapota* leaves, British Honduras," *Bull. Torr. Bot. Club*, 1944, **71** (3), 235–45.

9. Frear, D. H. .. "Photoelectric apparatus for measuring leaf areas," *Plant Physiol.*, 1935, **10** (3), 569–74.

10. Gerdel, R. W. and Salter, R. M. "Measurement of leaf area using the photoelectric cell," *Jour. Amer. Soc. Agron.*, 1928, **20** (6), 635–42.

11. Goodall, D. W. .. "Diurnal changes in the area of Cacoa leaves," *Ann. Bot., N. S.*, 1947, **11**, 449–51.

12. Gregory, F. G. .. "Studies in the energy relations of plants. I. The increase in area of leaves and leaf surface of *Cucumis sativus*," *Ann. Bot.*, 1921, **35**, 94.

13. Gustafson, F. G. and Elnore, S. "Some relations between leaf area and fruit size in tomatoes," *Plant Physiol.*, 1936, **11** (2), 445–51.

14. Hercik, F. .. "The relation between the surface tension of the sap and the area of plant leaf," *Biol. Listy.*, 1927, **13** (5/6), 390–99.

15. Hibbard, R. P., Grigsby, B. H. and Keck, W. G. "A low light intensity photoelectric device for the measurement of leaf areas," *Papers. Michigen. Acad. Sci. Arts. and Math.*, 1937–38, **23**, 141–47.

16. Hopkins, J. W. .. "Estimation of leaf area in wheat from linear dimensions," *Canad. Jour. Res. Sect. C. Bot. Sci.*, 1939, **17** (9), 300–04.

17. Kelly, V. W. .. "Relation of leaf form to transpiration rate and drought resistance in some deciduous fruits," *Proc. Amer. Soc. Hort. Sci.*, 1938–39, **36**, 210–15.

18. Khanna, K. L. .. *Annual Report of the Sugarcane Research Scheme in Bihar.* Supt. Govt. Printing Bihar, Patna, 1943.

19. Klages, K. H. W. .. "Metrical attributes and the physiology of hardy varieties of winter wheat," *Jour. Amer. Soc. Agron.*, 1926, **18** (7), 529–66.

20. Lal, K. N. and Mehrotra, O. N. "On some physiological attributes of transpiring surface in relation to drought resistance in sugarcane," *Proc. Ind. Acad. Sci.*, B, 1950 (Accepted).

21. Marshall, R. E. .. "An apparatus for ready determination of areas of compound leaves," *Jour. Agri. Res.*, 1934, **47** (6), 437–74.

22. Martin, J. H. .. "The comparative drought resistance of Sorghum and corn," *Jour. Amer. Soc. Agron.*, 1930, **22** (12), 993–1003.

23. Mathur, R. N. .. "Analysis of certain varietal characteristics in sugarcane with reference to drought resistance and yield," *Proc. Ind. Sci. Cong.*, 1938, 215.

24. McCrory, S. A. .. "Heat and drought tolerance of sweet co n as influenced by tillers," *Proc. Amer. Soc. Hort. Sci.*, 1942 (43), **40**, 424–28.

25. Morton, A. G. and Watson, D. J. .. "A physiological study of leaf growth," *Ann. Bot.*, (*N. S.*), 1948, **12**, 281–310.

26. Murneek, A. E. .. "Relation of leaf area to fruit size and food reserves in apple seeds and branches," *Proc. Amer. Soc. Hort. Sci.*, 1932 (33), **29**, 230–34.

27. Oinoue, Y. .. "A new mathematical method of measuring grape leaves," *Jour. Sci. Agric. Soc.*, 1927, **300**, 525–34.

28. Papen, R. V. .. "Beitrage zur Kenntnis des Waschstums der Blattspreite," *Bot. Arch. (Leipzig)*, 1935, **27** (2), 159–206.

29. Paterson, D. D. .. *Statistical Technique in Agricultural Research.* McGraw-Hill Book Company, New York, 1939, 107–09.

30. Pickett, W. F. .. "Leaf area in relation to apple production," *Bienn. Rept. Kansas State. Hort. Soc.*, 1934, **42**, 107–11.

31. Potter, G. F. .. "Photoelectric measurement of apple leaf areas," *Proc. Amer. Soc. Hort. Sci.*, 1937 (38), **35**, 260–62.

32. Romsher, F. A. .. "Correlation between fresh weight and area of tomato leaves," *Ibid.*, 1942 (43), **40**, 482.

33. Takasaki, T. .. "On relation between character and productivity proper to the varieties of potatoes," *Ann. Agric. Exp. Stat. Govt. Gen. Chosen*, 1929, **4** (4), 209–20.

34. Takiguti, Y. .. "Effects of reducing the leaf area on the yield and quality of rice," *Bulteno Sci. Fakult. Terkult. Kjusu Imp. Univ. Fukuoka, Japan*, 1929, **3** (4), 350–57.

35. Tiren, L. .. "The needle-area of pine stands," *Medd. Statens Skogst. Aust.*, 1926, **23**, 295–330.

36. Turrel, F. M. .. "The area of the internal exposed surface of dicotyledon leaves," *Amer. Jour. Bot.*, 1936, **23** (4), 255–64.

37. Watson, D. J. .. "The estimation of leaf area in field crops," *Jour. Agric. Sci.*, 1937, **27**, 474–83.

38. ———— .. "Comparative physiological studies on the growth of field crops. I. Variation in net assimilation rate and leaf area between species and varieties and within and between years," *Ann. Bot.* (*N.S.*), 1947, **11**, 41–76.

39. ———— .. "Comparative physiological studies on the growth of field crops. II. The effect of varying nutrient supply on net assimilation rate and leaf area," *Ibid.*, 1947, **11**, 375–407.

40. Withrow, R. B. .. "A photoelectric device for the rapid measurement of leaf area," *Jour. Agric. Res.*, 1935, **50** (7), 637–43.

# SAMPLING STUDIES IN SUGARCANE

## IV. The Estimation of Fibre Content

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### I. Introduction

Fibre is the dry water-insoluble matter of sugarcane and it greatly influences the milling house efficiency, *viz.*, extraction, milling capacity, recovery of commercial sugar, etc. A suitable sampling technique for its accurate estimation is thus a desideratum. That a slight increase in fibre per cent. of cane brings about considerable reduction in recoverable 96° sugar has been shown by Kerr and Cassidy (1937) and Daubert (1939). Davis (1936) and Ryan (1937) drew attention to the laboratory technique for the determination of fibre content. Earlier Moberly (1935) had pointed out that the accuracy of any method of determining fibre depends primarily upon the efficiency of sampling and that reasonably consistent results might be obtained with samples of 200 pounds of cane. Kerr (1939) in his investigations on the sampling studies found that there was a considerable amount of variation in fibre content within the stalk, from stalk to stalk, and between different varieties. He showed, however, that a sample of 18 stalks for 'Badila', 11 for "Clark's seedling", 14 for "S. J. 4" and 43 for "D 1135" was sufficient for the reliable estimation of fibre content.

The determination of fibre of a complete stalk affords practical difficulties and it is, therefore, imperative to see if a whole stalk could be replaced by some of its specific portions. The aim of the present contribution is to present systematically the variations that exist in between rows, canes and portions of a cane and to indicate, following a study of the variations, the minimum sample size required for a plot of an 1/40th acre in the accurate assessment of fibre value.

### II. Method of Fibre Determination Used in the Laboratory

The standard direct method (Appendix) recommends the use of calico bags for washing the pulp but results obtained at the station (Table I) showed

that fibre content varied appreciably when fresh and used calico bags were employed, thus necessitating their frequent changes and maintenance of quality. Besides such a method would be of little avail where large rounds of analysis have to be completed during the course of the day.

TABLE I

*Showing fibre % cane in fresh and used calico bags*

| Sl. No. | Progressive estimations with the same Calico bags | Fibre per cent. stalk as determined by direct method | |
|---|---|---|---|
| | | Co 313 | Co 299 |
| 1 | Fresh .. | 15·94 | 17·97 |
| 2 | One .. | 15·75 | 18·14 |
| 3 | Two .. | 15·90 | 17·98 |
| 4 | Three .. | 15·82 | 17·78 |
| 5 | Four .. | 15·70 | 17·79 |
| 6 | Five .. | 15·85 | 17·82 |
| 7 | Six .. | 15·62 | 17·68 |
| 8 | Seven .. | 15·40 | 17·44 |
| 9 | Eight .. | 15·34 | 17·52 |
| 10 | Nine .. | 15·00 | 17·25 |
| 11 | Ten .. | 14·84 | 17·05 |
| 12 | Eleven .. | 14·65 | 16·88 |
| 13 | Twelve .. | 14·36 | 16·90 |
| 14 | Thirteen .. | 14·10 | 16·72 |
| 15 | Fourteen .. | 13·98 | 16·65 |
| 16 | Fifteen .. | 13·90 | 16·50 |

Recourse had, therefore, to be had to Deerr's indirect method (1928). Each sampling unit was shredded and pulp obtained by means of an electric defibrating machine (Plate VIII). Precautions were taken to keep the pulp covered till weighment which was done almost immediately after defibration in order to reduce to minimum the loss of moisture due to evaporation. It was then dried in an electric oven at 102° C. to constant weight. The fibre per cent. was calculated with the help of the formula,

$$\text{Fibre per cent. of cane} = 100 - \frac{100\,m}{100 - b},$$

where $m$ = moisture per cent. pulp,

$b$ = brix per cent. cane which was found from sampling units

by crushing the remaining portions of the canes. That this method gives reliable results will be obvious from data (Table II) collected with the two methods.

TABLE II

*Showing comparative results by two methods of fibre determination*

| Sl. No. | Fibre % cane | | Sl. No. | Fibre % cane | |
|---|---|---|---|---|---|
| | Direct | Indirect | | Direct | Indirect |
| 1 | 16·67 | 16·87 | 46 | 12·20 | 12·10 |
| 2 | 15·80 | 15·40 | 47 | 15·40 | 14·37 |
| 3 | 13·80 | 14·12 | 48 | 11·20 | 10·60 |
| 4 | 14·20 | 13·60 | 49 | 16·40 | 16·56 |
| 5 | 13·60 | 14·01 | 50 | 16·20 | 16·44 |
| 6 | 13·40 | 12·57 | 51 | 15·60 | 15·61 |
| 7 | 17·80 | 17·36 | 52 | 15·80 | 15·88 |
| 8 | 13·60 | 13·14 | 53 | 15·80 | 16·48 |
| 9 | 14·20 | 13·00 | 54 | 16·80 | 17·13 |
| 10 | 13·60 | 14·07 | 55 | 16·60 | 16·22 |
| 11 | 15·40 | 18·52 | 56 | 15·40 | 14·98 |
| 12 | 14·20 | 13·63 | 57 | 16·80 | 16·91 |
| 13 | 16·80 | 16·56 | 58 | 16·00 | 16·29 |
| 14 | 13·60 | 13·06 | 59 | 16·60 | 16·40 |
| 15 | 12·60 | 12·14 | 60 | 16·40 | 15·89 |
| 16 | 10·20 | 10·00 | 61 | 13·44 | 14·44 |
| 17 | 14·20 | 13·86 | 62 | 13·80 | 13·44 |
| 18 | 15·40 | 14·96 | 63 | 13·60 | 14·52 |
| 19 | 16·60 | 17·23 | 64 | 15·40 | 15·87 |
| 20 | 15·20 | 15·67 | 65 | 14·80 | 14·34 |
| 21 | 14·00 | 14·14 | 66 | 16·60 | 15·69 |
| 22 | 14·20 | 13·22 | 67 | 16·80 | 16·79 |
| 23 | 13·70 | 13·96 | 68 | 16·40 | 16·31 |
| 24 | 10·60 | 13·83 | 69 | 15·60 | 16·04 |
| 25 | 12·60 | 13·10 | 70 | 16·20 | 16·56 |
| 26 | 12·60 | 12·48 | 71 | 16·80 | 16·04 |
| 27 | 14·00 | 13·90 | 72 | 15·60 | 15·42 |
| 28 | 12·00 | 13·32 | 73 | 18·00 | 17·85 |
| 29 | 14·20 | 13·30 | 74 | 16·60 | 16·51 |
| 30 | 15·20 | 15·06 | 75 | 13·80 | 13·88 |
| 31 | 14·60 | 14·76 | 76 | 15·60 | 14·96 |
| 32 | 14·40 | 13·94 | 77 | 14·60 | 14·16 |
| 33 | 14·20 | 14·35 | 78 | 13·80 | 14·04 |
| 34 | 15·00 | 16·34 | 79 | 14·40 | 13·36 |
| 35 | 14·45 | 14·16 | 80 | 12·4 | 11·02 |
| 36 | 15·70 | 14·96 | 81 | 14·40 | 14·43 |
| 37 | 17·20 | 17·80 | 82 | 14·60 | 14·52 |
| 38 | 14·20 | 14·45 | 83 | 14·40 | 13·78 |
| 39 | 14·40 | 14·74 | 84 | 16·50 | 15·52 |
| 40 | 15·60 | 16·13 | 85 | 14·00 | 15·20 |
| 41 | 16·00 | 15·72 | 86 | 15·00 | 14·24 |
| 42 | 15·20 | 15·38 | 87 | 17·60 | 17·18 |
| 43 | 13·20 | 12·26 | 88 | 14·00 | 14·41 |
| 44 | 12·40 | 12·48 | 89 | 15·20 | 14·98 |
| 45 | 13·20 | 13·40 | 90 | 14·00 | 14·79 |

The analysis of variance (Table III) showed that differences due to methods were statistically not significant and that the indirect method could be reliably employed for studies of this nature.

TABLE III

*Showing analysis of variance*

| Due to | Degrees of freedom | Sum of squares | Variance | Ratio |
|---|---|---|---|---|
| Between methods .. | 1 | 0·1212 | 0·1212 | 0·0455 |
| Within methods (Error) .. | 178 | 474·4926 | 2·6657 | |
| Total .. | 179 | 474·6138 | | |
| Mean Fibre % (Direct) .. | 14·79 | | | |
| Mean Fibre % (Indirect) .. | 14·73 | | | |

## III. EXPERIMENTAL DATA

With the help of the formula outlined in the preceding section, the data on fibre per cent. have been collected for three varieties—Co 299, Co 313 and Co 356 representing early, medium-early and mid-season varieties. The material falls under three categories. In the first category rows were at first selected at random in a plot and then random selection was made of canes in each row in such a manner as not to include two canes from the same clump. Each cane was subsequently divided into three approximately equal divisions (1) bottom, (2) middle and (3) top; and each division was further subdivided into portions, each containing a complete nodal length (a node and an internode). Two such portions in a division were separately analysed for fibre content. For each cane, therefore, there were six observations, each being the value of fibre per cent. of a portion. The statement below gives the details of the data collected under the first category:

| Sl. No. | Variety | No. of plots | Size of the plot | Time of fibre determination | No. of sampling units in | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Rows | Canes | Div. | Portions |
| i (*a*) | Co. 313 | 1 | 1/40th acre (40′ × 27′) | November | 10 | 10 | 3 | 2 |
| i (*b*) | Co. 313 | 6 | 1/40th acre (60′ × 21′) | February | 3 | 15 | 3 | 2 |
| i (*c*) | Co. 299 | 4 | 1/40th acre (60′ × 21′) | March | 3 | 15 | 3 | 2 |

Under the second category, fibre per cent. was determined individually for each cane available in two plots (1/40th acre each),—one under Co 313

and the other under Co 299. The details of data under the third category, where the fibre per cent. was determined clumpwise are given below:

| Sl. No. | Veriety | No. of plots | Size of the plot | Time of fibre determination | No. of sampling unit in | |
|---|---|---|---|---|---|---|
| | | | | | Rows* | Clumps |
| iii (*a*) | Co. 313 .. | 4 | 1/40th acre (60′ × 21′) | January | 6 | 10 |
| iii (*b*) | Co. 313 .. | do | do | March | do | do |
| iii (*c*) | Co. 299 .. | do | do | January | do | do |
| iii (*d*) | Co. 299 .. | do | do | March | do | do |
| iii (*e*) | Co. 356 .. | do | do | January | do | do |
| iii (*f*) | Co. 356 .. | do | do | March | do | do |

* Central 2-7 rows were taken.

## IV. Nested Sampling

Formulæ of Nested sampling (Roy and Banerjee, 1940 and Mahalanobis, 1944) have been adopted in order to get the estimates of the variations contributed by the successive stages of sampling—plots, rows, canes divisions and portions, which have been defined as the zones of successive orders. $\sigma_1^2$, $\sigma_2^2$, $\sigma_3^2$, $\sigma_4^2$ and $\sigma_5^2$, the true variances in the respective zones of the five-fold Nested sampling (equal case) may be estimated in the following manner:

Let

$v_1$ be the mean square between plots
$v_2$ ,, ,, rows within plots
$v_3$ ,, ,, canes within rows
$v_4$ ,, ,, divisions within canes
$v_5$ ,, ,, portions within divisions

Then

$v_5$ will estimate $\sigma_5^2$
$v_4$ ,, $m\sigma_4^2+\sigma_5^2$
$v_3$ ,, $mn\sigma_3^2+m\sigma_4^2+\sigma_5^2$
$v_2$ ,, $mnp\sigma_2^2+mn\sigma_3^2+m\sigma_4^2+\sigma_5^2$
$v_1$ ,, $mnpq\sigma_1^2+mnp\sigma_2^2+mn\sigma_3^2+m\sigma_4^2+\sigma_5^2$

The variance of the mean would be given by:

$$v(x.....) = \frac{\sigma_1^2}{t} + \frac{\sigma_2^2}{tq} + \frac{\sigma_3^2}{tqp} + \frac{\sigma_4^2}{tqpn} + \frac{\sigma_5^2}{tqpnm}$$

$t$ is the number of plots

$q$ ,, rows in a plot

$p$ ,, canes in a row

$n$ ,, divisions in a cane

$m$ ,, portions in a division.

The formulæ corresponding to Nested sampling of lesser orders, used in course of the present paper, will follow from symmetry.

(ii) The formulæ for three-fold Nested sampling in unequal case will also be required. They are reproduced below:

Let $v_1$, $v_2$ be respectively the mean squares due to the zones of successive orders (*e.g.*, rows, clumps and canes) in the table of Analysis of variance. Then $\sigma_1^2$, $\sigma_2^2$, $\sigma_3^2$, the true variances in the zones of successive orders may be estimated as follows:

$v_3$ will estimate $\sigma_3^2$

$(n-t)\,v_2$ will estimate $\left\{m - \Sigma_i \frac{\Sigma_j m_{ij}^2}{m_i}\right\} \sigma_2^2 + \sigma_3^2 (n-t)$

$(t-1)\,v_1$ will estimate $\left\{m - \frac{\Sigma_i m_i^2}{m}\right\} \sigma_1^2 + \left\{\Sigma_i \frac{\Sigma_j m_{ij}^2}{m_i} - \frac{\Sigma_i \Sigma_j m_{ij}^2}{m}\right\}$

$$\sigma_2^2 + \sigma_3^2 (t-1),$$

where

$m_{ij}$ = the number of third order zones in the $j$th second order zone of the $i$th first order zone.

$m_i$ = the total number of third order zones occurring in the $i$th first order zone, and

$\Sigma_i m_i = m$ = the total number of third order zones.

$n$ = the total number of second order zones.

$t$ = the total number of first order zones.

Finally

$$v(x\ldots.) = \frac{\Sigma_i m_i^2}{m^2}\sigma_1^2 + \frac{\Sigma_i \Sigma_j m_{ij}^2}{m^2}\Sigma_2^2 + \frac{1}{m}\sigma_3^2$$

If in the process of estimation, the estimate of any variance comes out to be negative, it has to be taken as zero.

## V. Variations Occurring in Portions and Divisions as Against Canes and Rows

### (*i*) *Co* 313

The data (*ia*) mentioned in section (3) consists of $10 \times 10 \times 3 \times 2 = 600$ observations, each being the value of fibre per cent. of a portion in a cane. There are 100 canes in all and for each cane there are 6 such observations. Using the formulæ corresponding to four-fold Nested sampling, the estimates of the variances due to rows, canes, divisions and portions have been obtained and shown in the last line of Table IV. Columns (5), (7), (9) and (11) of the table show respectively these estimates and the adjacent columns the percentage variations due to each of these four sources. It will be seen that the variations due to canes is the maximum being 40·63% and that due to rows is the minimum being 4·02%. The percentage variations due to divisions and portions are 37·77 and 17·58 respectively. The mean fibre per cent. is 14·09 and the error expressed as its percentage has come out to be 1·35.

Fibre per cent. for this plot was determined during the month of November, whereas the fibre per cent. for each of the 6 plots shown in the first 6 rows of Table IV was determined during February. For the purpose of comparing the errors in these two sets of plots, account has to be taken of the fact that the sample sizes were different. The average error % in the first 6 plots is 2·91, whereas the adjusted error per cent. for the 7th plot, in the table, comes out to be 2·21. For all practical purposes, these two percentages may be considered to be of the same order. The average fibre per cent. for the first 6 plots is 13·67, whereas the fibre per cent. for the 7th plot is 14·09. These values also are not significantly different from one another. The variations due to the different sources in these 6 plots show more or less a similar trend with those of the last plot.

When, however, a pooled analysis is made of the first 6 plots and the estimates of the variances obtained by the formulæ given in Section (4) it is seen that variation due to canes still remains the maximum and that the

## TABLE IV

*Estimates of the variances due to the different zones and the S.E. expressed as percentage of the mean*

Co 313 (Phattak) (February)

| | Mean | $\sigma_m$ | $\sigma_m$% mean | $\sigma_1^2$ | $\sigma_1^2$% of total | $\sigma_2^2$ | $\sigma_3^2$% of Total | $\sigma_3^2$ | $\sigma_3^2$% of Total | $\sigma_4^2$ | $\sigma_4^2$% of Total | Plot size | Observations |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| Plot 1 .. | 14·49 | 0·49 | 3·38 | 0·5589 | 13·72 | 1·9974 | 49·02 | 0·8150 | 20·00 | 0·7034 | 17·26 | 1/40 acre | 3×15×3×2 |
| 2 .. | 14·04 | 0·41 | 2·72 | 0·3507 | 8·97 | 1·8950 | 48·72 | 0·8950 | 22·82 | 0·7636 | 19·49 | do | do |
| 3 .. | 12·80 | 0·28 | 2·19 | 0·0807 | 1·05 | 1·2775 | 16·82 | 0 | 0 | 6·2550 | 82·13 | do | do |
| 4 .. | 13·79 | 0·31 | 2·29 | 0·1176 | 3·15 | 2·2167 | 89·32 | 0·4575 | 12·24 | 0·9451 | 25·29 | do | do |
| 5 .. | 14·14 | 0·42 | 2·97 | 0·3899 | 10·37 | 1·8017 | 47·87 | 0·6150 | 16·50 | 0·9479 | 25·26 | do | do |
| 6 .. | 12·75 | 0·47 | 3·69 | 0·4029 | 6·59 | 3·3267 | 54·86 | 1·5950 | 26·36 | 0·7444 | 12·19 | do | do |
| Co. 313 (Dhab) (November) | | | | | | | | | | | | | |
| 7 .. | 14·09 | 0·19 | 1·35 | 0·1464 | 4·02 | 1·4797 | 40·63 | 1·3754 | 37·77 | 0·6403 | 17·58 | 1/40 acre | 10×10×3×2 |

$\sigma_1^2$ = Variance due to Rows $\sigma_2^2$ = Variance due to Canes
$\sigma_3^2$ = ,, ,, ,, Divisions $\sigma_4^2$ = ,, ,, ,, Portions

variation due to plots now becomes the minimum. The following table gives the estimated variances and their values expressed as percentages of the total variation:

| | Variance | Variance % of total |
|---|---|---|
| Portions .. | 0·2334 | 21·10 |
| Divisions .. | 0·2018 | 18·35 |
| Canes .. | 0·3313 | 30·28 |
| Rows .. | 0·2108 | 19·27 |
| Plots .. | 0·1226 | 11·00 |

(*ii*) *Co* 299

The similar data available for Co 299 have been subjected to the same type of analysis and the results have been set out in Table V. It has been found that the mean fibre per cent. of Co 299 is significantly higher than that of Co 313. The variations in the fibre per cent. shown by the different sources are in general high and have reflected in the high standard errors. In one plot, the standard error has been as high as 9·02%. These plots have shown a divergence amongst themselves in respect of the variances due to the different sources, but the variation due to canes has none the less been high in all the plots except in one.

This set of four plots has also been subjected to a pooled analysis. The estimates of the variances are given below:

| | Variance | Variance % of total |
|---|---|---|
| Portions .. | 0·6551 | 14·2875 |
| Divisions .. | 1·2805 | 26·3571 |
| Canes .. | 0·9983 | 21·7727 |
| Rows .. | 1·6612 | 36·2304 |
| Plots .. | 0·0620 | 1·3525 |

It will be noticed in the above table that the variation due to rows has been the highest and that due to divisions has been second in order of magnitude. The plots under Co 299, therefore, show a marked divergence

TABLE V

*Estimates of the variance due to the different zones and the S. E. expressed as percentage of the mean*

Co 299 (Nepali) (March)

| | Mean | $\sigma_m$ | $\sigma_m$% mean | $\sigma_1^2$ | $\sigma_1^2$% of Total | $\sigma_2^2$ | $\sigma_2^2$% of Total | $\sigma_3^2$ | $\sigma_3^2$% of Total | $\sigma_4^2$ | $\sigma_4^2$% of Total | Plot size | Observation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| Plot 1 .. | 16·04 | 0·35 | 2·18 | 0·1322 | 2·23 | 2·0640 | 34·81 | 2·6431 | 44·57 | 1·0903 | 18·39 | 1/40 acre | 3×15×3×2 |
| 2 .. | 17·41 | 1·57 | 9·02 | 6·8774 | 39·62 | 6·4822 | 37·34 | 0·6892 | 3·97 | 3·3116 | 19·07 | do | do |
| 3 .. | 16·87 | 0·95 | 5·63 | 2·0851 | 16·47 | 8·5079 | 67·23 | 1·0135 | 8·01 | 1·0488 | 8·29 | do | do |
| 4 .. | 14·98 | 0·77 | 5·14 | 0·9251 | 3·14 | 5·3698 | 18·24 | 22·8948 | 77·75 | 0·2574 | 0·87 | do | do |

$\sigma_1^2$ = Variances due to Rows $\sigma_2^2$ = Variances due to Canes
$\sigma_3^2$ = ,, ,, ,, Divisions $\sigma_4^2$ = ,, ,, ,, Portions

from the plots under Co 313, analysed before, in the distribution of the percentage varations.

## VI. Agreement In Between the Fibre Per Cent. Values Obtained from a Composite Sample of a Cane and the Mean of the Fibre Per Cent. Values Obtained from Sub-Samples of the Cane

An attempt has then been made to find out whether or not the fibre per cent. obtained from a composite sample of a number of sub-samples taken from a cane agrees with the mean fibre per cent. of the sub-samples. For this purpose, 12 random canes of Co 313 were taken and each was divided into 6 portions. The fibre per cent. was then determined for each composite sample as well as individually for each sub-sample. Another set of 12 random canes was taken and each cane was divided into 3 portions for similar analysis. Table VI shows these details. Student's *t*-tests reveal

Table VI

*Showing the agreement in between the mean of individual values and the value of the composite sample*

| | Set I | | Set II | |
|---|---|---|---|---|
| | Mean of individual values | Value of composite sample | Mean of individual values | Value of composite sample |
| 1 | 13·99 | 13·51 | 13·53 | 13·44 |
| 2 | 12·88 | 13·33 | 15·43 | 14·98 |
| 3 | 12·80 | 13·73 | 12·85 | 12·32 |
| 4 | 13·80 | 13·24 | 15·09 | 15·38 |
| 5 | 12·58 | 13·08 | 14·29 | 14·62 |
| 6 | 12·66 | 12·90 | 13·98 | 15·52 |
| 7 | 12·14 | 12·66 | 14·86 | 16·03 |
| 8 | 13·89 | 15·15 | 13·81 | 14·38 |
| 9 | 11·01 | 11·49 | 15·91 | 15·74 |
| 10 | 11·80 | 12·13 | 12·73 | 13·43 |
| 11 | 12·12 | 12·29 | 14·82 | 15·54 |
| 12 | 13·11 | 13·55 | 13·08 | 14·37 |
| | Mean difference = 0·27 | | Mean difference = 0·44 | |

that the agreement in between the mean of the individual values and the value of the composite sample is very close in both the sets.

## VII. Data Collected under the Second Category Co 313 and Co 299

After having arrived at the conclusion that the fibre per cent. of a cane determined from a composite sample can be relied upon, it was decided

to determine on this basis, the fibre per cent. of all the canes in an 1/40th acre plot under each of the varieties, Co 313 and Co 299. There were 7 rows, 242 clumps and 567 canes in the plot under Co 313, and 7 rows, 220 clumps and 671 canes in the plot under Co 299. The estimation of the variances due to rows ($\sigma_1^2$), clumps ($\sigma_2^2$) and canes ($\sigma_3^2$) obtained according to the formulæ (unequal numbers) outlined in Section 4 (ii) are given below:

| Variety | Mean | $\sigma_m$ | $\sigma_m$% of mean | $\sigma_1^2$ | $\sigma_1^2$% of total | $\sigma_2^2$ | $\sigma_2^2$% of total | $\sigma_3^2$ | $\sigma_3^2$% of total |
|---|---|---|---|---|---|---|---|---|---|
| Co. 313 .. | 14·21 | 0·52 | 3·65 | 1·75 | 18·27 | 0 | 0 | 7·83 | 81·73 |
| Co. 299 .. | 18·32 | 0·47 | 2·57 | 1·39 | 12·29 | 3·50 | 30·67 | 6·52 | 57·14 |

Here also, it is found that the mean fibre per cent. of Co 299 is significantly higher than that of Co 313. It will be noticed further that the percentage variation due to canes ($\sigma_3^2$) has been the highest. The striking feature in the above results appears to be the high magnitudes of the standard errors. The error in respect of Co 313 has been as high as 3·65%, which seems to be rather too high for the data of complete enumeration.

A random sample taken from these data has also been analysed. Ten clumps were selected at random in each of the seven rows and the fibre per cent. values of two canes taken at random from each clump were subjected to similar analysis. The estimates of the variances due to rows, clumps and canes are given below:

| Variety | Mean | $\sigma m$ | $\sigma m$% of total | $\sigma_1^2$ | $\sigma_1^2$% of total | $\sigma_2^2$ | $\sigma_2^2$% of total | $\sigma_3^2$ | $\sigma_3^2$% of total |
|---|---|---|---|---|---|---|---|---|---|
| Co 313 .. | 14·18 | 0·40 | 2·82 | 1·09 | 12·51 | 4·41 | 50·84 | 3·18 | 36·65 |
| Co 299 .. | 19·03 | 0·54 | 2·84 | 2·54 | 20·00 | 5·10 | 40·00 | 5·09 | 40·00 |

The differences in the mean fibre per cent. values obtained from the complete data and the sample sets are not statistically significant and both these sample sets have also revealed that canes contribute a considerable part of the total variation. The error in respect of Co 313 in the sample set has been less than that in the data of complete enumeration; and it is mainly due to the fact that $\sigma_2^2$ came out to be zero in the latter.

## VIII. Data Collected Under the Third Category

Fibre per cent. was determined during January and March for a set of 4 plots each, under each of the varieties, Co 313, Co 299 and Co 356, where

the fibre per cent. of a clump was found from a composite sample. The estimated variance with other details have been set out in Table VII.

TABLE VII

*Estimates of the variances due to the different zones and S. E. expressed as percentage of the mean*

| | Mean | $\sigma_m$ | $\sigma_m$% of mean | $\sigma_1^2$ | $\sigma_1^2$% Total | $\sigma_2^2$ | $\sigma_2^2$% Total | Plot size | No. of observations |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Co 313 (January data) | | | | |
| Plot 1 | 13·13 | 0·24 | 1·83 | 0·00 | 0·00 | 3·5422 | 100·00 | 1/40 acre | 6 rows × 10 clumps |
| 2 | 13·16 | 0·17 | 1·29 | 0·00 | 0·00 | 1·7572 | 100·00 | do | do |
| 3 | 13·51 | 0·20 | 1·48 | 0·00 | 0·00 | 2·4098 | 100·00 | do | do |
| 4 | 13·62 | 0·25 | 1·84 | 0·1522 | 6·04 | 2·3681 | 93·96 | do | do |
| | | | | | Co 299 (January data) | | | | |
| Plot 1 | 15·84 | 0·22 | 1·39 | 0·4208 | 13·14 | 2·7820 | 86·86 | do | do |
| 2 | 16·70 | 0·47 | 2·81 | 1·0495 | 29·44 | 2·5148 | 70·56 | do | do |
| 3 | 15·13 | 0·37 | 2·45 | 0·5442 | 15·44 | 2·9580 | 84·46 | do | do |
| 4 | 15·79 | 0·37 | 2·34 | 0·5384 | 16·20 | 2·7856 | 83·80 | do | do |
| | | | | | Co 356 (January data) | | | | |
| Plot 1 | 14·76 | 0·39 | 2·64 | 0·5899 | 15·48 | 3·2213 | 84·52 | do | do |
| 2 | 14·97 | 0·24 | 1·60 | 0·00 | 0·00 | 3·5296 | 100·00 | do | do |
| 3 | 14·70 | 0·31 | 2·11 | 0·2190 | 5·83 | 3·4903 | 94·17 | do | do |
| 4 | 15·16 | 0·24 | 1·58 | 0·00 | 0·00 | 3·4985 | 100·00 | do | do |
| | | | | | Co 313 (March data) | | | | |
| Plot 1 | 14·85 | 0·21 | 1·41 | 0·1074 | 6·78 | 1·4773 | 93·22 | do | do |
| 2 | 15·31 | 0·26 | 1·70 | 0·00 | 0·00 | 3·9859 | 100·00 | do | do |
| 3 | 16·34 | 0·21 | 1·29 | 0·1343 | 9·28 | 1·3133 | 90·72 | do | do |
| 4 | 15·75 | 0·26 | 1·65 | 0·2946 | 20·35 | 1·1530 | 79·65 | do | do |
| | | | | | Co 299 (March data) | | | | |
| Plot 1 | 18·67 | 0·31 | 1·66 | 0·4720 | 26·38 | 1·3171 | 73·62 | do | do |
| 2 | 18·07 | 0·25 | 1·38 | 0·2180 | 12·19 | 1·5701 | 87·81 | do | do |
| 3 | 19·23 | 0·20 | 1·04 | 0·00 | 0·00 | 2·3975 | 100·00 | do | do |
| 4 | 17·66 | 0·21 | 1·19 | 0·0685 | 3·57 | 1·9031 | 96·43 | do | do |
| | | | | | Co 336 (March data) | | | | |
| Plot 1 | 19·20 | 0·25 | 1·30 | 0·1647 | 6·64 | 2·3153 | 93·36 | do | do |
| 2 | 17·67 | 0·51 | 2·89 | 1·0434 | 16·10 | 5·4371 | 83·90 | do | do |
| 3 | 17·59 | 0·32 | 1·82 | 0·0884 | 1·64 | 5·3092 | 98·36 | do | do |
| 4 | 19·44 | 0·17 | 0·87 | 0·0632 | 5·42 | 1·1024 | 94·58 | do | do |

$\sigma_1^2$ = Variation due to Rows. $\sigma_2^2$ = Variation due to Clumps.

Analysis reveals that the mean fibre per cent. in January was significantly less than the mean fibre per cent. in March, whereas in between the different

varieties themselves, the mean fibre per cent. shows the following order of magnitude:—

January—Co 299 > Co 356 > Co 313

March—Co 299 = Co 356 > Co 313.

The standard errors expressed as percentages have been very low for all the varieties. The table will indicate that in majority of the plots the standard errors have been well within 2%. It will further be noticed that the variation between rows is but insignificant compared to the variation between clumps.

## IX. Sampling Requirements

On the basis of the standard errors obtained in the different plots, it is now proposed to find out the required sampling size. Error in all the

### Table VIII

*Number of canes to be selected from each row so that the S.E. may not exceed 2½% and 5% of the mean*

*Results for plots analysed in Table IV*

Rows = 3

| | Plot I | Plot II | Plot III | Plot IV | Plot V | Plot VI | Plot VII | Remarks |
|---|---|---|---|---|---|---|---|---|
| 5% Co. 313 .. | 3 | 3 | 3 | 2 | 2 | 5 | $r = 2, c = 3$<br>$r = 3, c = 2$ | In the first 6 plots rows = 3 |
| 2½% Co. 313 .. | * | Very large | 11 | 11 | * | * | $r = 3, c = 10$<br>$r = 2, c = 20$ | Divisions = 3 in a cane and portions = 6 in a cane |

*Results for plots analysed in Table V*

| | 5% | | | | 2½% | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Plot I | Plot II | Plot III | Plot IV | Plot I | Plot II | Plot III | Plot IV | Remarks |
| Co. 299 .. | 2 | * | Approx. 15 | Approx. 15 | 9 | * | * | * | Rows = 3<br>Divisions = 3 in a cane<br>Portions = 6 in a cane |

* Indicates "*Impossible*"

plots for Co 313, Co 356 and Co 299 analysed in Tables IV and V have been less than 5% of the mean except in three plots under Co 299 (*vide* Table V). In the data referred to in these two tables, the fibre per cent. of a cane was represented by the fibre per cent. values of six portions taken equally from its three divisions, whereas in the data collected subsequently the fibre per cent. of a cane or of a clump was represented by the fibre per cent. value of a composite sample. For the sake of comparison with the results obtained in the subsequent plots it has been considered necessary to keep in the former plots the number of divisions and portions as they are in working out the sample sizes.

Since only three rows were taken at random from each of the plots analysed in Tables IV and V except for plot (7) in Table IV, the number of rows has not been increased beyond " three " in working out the sample sizes for these plots. In Table VIII have been shown the required samples sizes in terms of the number of canes per row worked out on the basis of 2½% and 5% error margin. In some plots, the contribution to the error per cent. made by rows has been too high to admit of the required reduction in error by an increase in the number of canes alone. Such cases have been marked with an asterisk(*). This contingency has occurred mostly under the error margin of 2½%, and brings out the necessity for a larger representation of rows in the random sample.

Similar combinations of rows, clumps and canes have also been worked out in respect of the random samples taken from the data of complete enumeration of Co 313 and Co 299. The table given below shows the combination worked out on the basis of 5% error. Combinations on the basis of 2½% error are not possible on account of the reasons furnished in the preceding paragraph.

| Co 313 | | | Co 299 | | |
|---|---|---|---|---|---|
| Rows | Clumps | Canes | Rows | Clums | Canes |
| 3 | 19 | 1 | 3 | 64 | 1 |
| 3 | 15 | 2 | 3 | 44 | 2 |
| 6 | 4 | 1 | 6 | 4 | 1 |
| 6 | 4 | 2 | 6 | 3 | 2 |

In the plots analysed in Tables IV and V, canes were selected in a row in such a manner that no two canes were taken from the same clumps,

TABLE IX

*Number of clumps to be selected from each row so that S.E. may not exceed 2½% and 5% of the mean*

*Results for plots analysed in Table VII*

*Data collected during January*

| | 5% | | | | 2½% | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Plot I | Plot II | Plot III | Plot IV | Plot I | Plot II | Plot III | Plot IV | Remarks |
| Co 313 .. | 3 | 2 | 2 | 2 | 12 | 6 | 7 | 11 | Rows = 3 |
| Co 299 .. | 2 | 3 | 3 | 3 | Very large | * | * | * | |
| Co 356 .. | 4 | 3 | 3 | 3 | * | 9 | 19 | 19 | |

*Data collected during March*

| | 5% | | | | 2½% | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Plot I | Plot II | Plot III | Plot IV | Plot I | Plot II | Plot III | Plot IV | Remarks |
| Co 313 .. | <1 | 3 | <1 | <1 | 5 | 10 | 4 | 7 | Rows = 3 |
| Co 299 .. | <1 | <1 | <1 | <1 | 8 | 4 | 4 | 4 | |
| Co 356 .. | <1 | 5 | 3 | <1 | 5 | * | 11 | 2 | |

*Results for plots analysed in Table VII*

*Data collected during January*

| | 5% | | | | 2½% | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Plot I | Plot II | Plot III | Plot IV | Plot I | Plot II | Plot III | Plot IV | Remarks |
| Co 313 .. | 2 | <1 | <1 | <1 | 6 | 3 | 4 | 5 | Rows = 6 |
| Co 299 .. | <1 | <1 | <1 | <1 | 6 | 10 | 10 | 10 | |
| Co 356 .. | 2 | 2 | 2 | 2 | 14 | 5 | 7 | 5 | |

*Data collected during March*

| | 5% | | | | 2½% | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Plot I | Plot II | Plot III | Plot IV | Plot I | Plot II | Plot III | Plot IV | Remarks |
| Co 313 .. | <1 | 2 | <1 | <1 | 3 | 5 | 2 | 2 | Rows = 6 |
| Co 299 .. | <1 | <1 | <1 | <1 | 2 | 2 | 2 | 2 | |
| Co 356 .. | <1 | 2 | 2 | <1 | 2 | 10 | 5 | 2 | |

* Indicates *Impossible*

Therefore, the results given in the first line of the above table might be compared with those set out in Table VIII.

In view of the large variations in individual canes due to differences in their ages it is necessary to take a clump, which represents all age groups so far as the progeny of a planted set is concerned, as a basis for recommending suitable sample size for fibre determination; and for this purpose the plots analysed in Table VII have been selected. Combinations of rows and clump have been worked out on the basis of 2½% and 5% errors and shown in Table IX where all the three varieties, *viz.*, early, medium-early and mid-season have been represented.

For an 1/40th acre plot, of which the number of rows would not exceed 6 or 7, the following sampling sizes may be recommended. Range of variation in the sizes has been indicated for each variety. The number of rows has been kept constant at 6, as it was pointed out before that rows should be given as large a representation as possible.

| | Error margin of 5% | | Error margin of 2½% | |
|---|---|---|---|---|
| | Rows × Clumps | = No. of Clumps | Rows × Clumps | = No. of Clumps |
| Co 313 .. | 6 × 1 | = 6 | 6 × 2 | = 12 |
| | 6 × 2 | = 12 | 6 × 6 | = 36 |
| Co 299 .. | 6 × 1 | = 6 | 6 × 2 | = 12 |
| | | | 6 × 10 | = 60 |
| Co 356 .. | 6 × 1 | = 6 | 6 × 2 | = 12 |
| | 6 × 2 | = 12 | 6 × 14 | = 84 |

The size of the sample has been shown in terms of the number of clumps, and it may be recalled here that the mean fibre per cent. value of a clump is to be determined from a composite sample taken from the different divisions and portions of a cane in the manner as described in Sub-Section VI.

## X. Summary of Conclusions

(1) Sampling studies were undertaken in order to evolve the minimum sample size for the estimation of fibre per cent. of sugarcane. Material in respect of Co 313, Co 299 and Co 356 has been examined in the present paper.

(ii) It has been revealed that the mean fibre per cent. in January is significantly less than the mean fibre per cent. in March. Further, the fibre per cent. of Co 313 is the least and that of Co 299 the highest. The fibre per cent. value of Co 356 lies between those of Co 313 and Co 299.

(iii) Analysis revealed that the fibre per cent. obtained from a composite sample taken from a cane agrees very closely with the mean of the fibre per cent. values of the different sub-samples comprising the composite sample.

(iv) Of the four sources of variation, namely rows, canes, divisions, and portions, the variation due to canes has been the maximum. This is understandable in view of the difference in the ages of the daughter shoots.

(v) A clump represents all age groups so far as the progeny of a planted set is concerned and therefore, sampling for fibre has to be confined to whole clumps drawn from different rows. For an 1/40th acre plot, the rows of which will not exceed 6 or 7, the required sammpling sizes with the rang of variation in each variety are indicated below. The fiber per cent. value of a clump has to be determined from a composite sample as outlined in Sub-Section VI.

| | Error margin of 5% | | Error margin of 2½% | |
|---|---|---|---|---|
| | Rows × Clumps | = No. of Clumps | Rows × Clumps | = No. of Clumps |
| Co 313 .. | 6 × 1 | = 6 | 6 × 2 | = 12 |
| | 6 × 2 | = 12 | 6 × 6 | = 36 |
| Co 299 .. | 6 × 1 | = 6 | 6 × 2 | = 12 |
| | | | 6 × 10 | = 60 |
| Co 356 .. | 6 × 1 | = 1 | 6 × 2 | = 12 |
| | 6 × 2 | = 12 | 6 × 14 | = 84 |

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## References

1. Daubert .. *Facts About Sugar*, 1939, **34**, **11**, 38–39.
2. Davis, M. B. .. *Proc. 2nd Ann. Conf. Q.S.S.C.T.*, 1936, 54–57.
3. Kerr, H. W. .. *I. S. J.*, 1939, **41**, **486**, 227–30.
4. ——— and Cassidy, N. G. .. *Bull. Bur. Sug. Expt. Sta.*, Queensland, 1937, **2**.
5. Mahalanobis, P. C. .. *Phil. Trans. Roy. Soc.*, London, 1944, **231**, 327–451.
6. Moberley, G. S. .. *Proc. 9th Cong. S. Afr. Sug. Technol. Assn.*, 1935, 61-64.
7. Deerr, Noel .. *Handbook Sug. Technol. Assn.*, India, 1928.
8. Ryan, W. J. .. *Proc. 3rd Ann. Conf. Q.S.S.C.T.*, 1937, 26–27.
9. Roy, S. N. and Banerjee, K. S. .. *Science and Culture*, 1940, **6**, **3**, 189.

## APPENDIX

### Determination of Fibre Content

*(Method Employed by Kerr and Cassidy)*

Sticks shall be taken from sample trucks in amounts approximating to the same weight from each such truck. When a class of a variety is estimated to constitute 50 per cwt. or more of the Cane supply, a determination shall be made daily, on five days of each work, when 25 per cent. to 50 per cent. of the supply, twice a week; when under 25 per cent. at least once a week. Provided that, when a class of variety is estimated to contribute less than 5 per cent. of the supply, a composite sample shall be made from all such varieties which shall be known as " MIXED ".

For the purpose of this regulation a " Class " shall be constituted by any one of the following: Green plant, green ratoons, burnt plant, or ratoons.

Alternate methods of sub-sampling shall be as follows:

(i) Sticks are arranged in order of descending length on the ground with tops all facing in own direction, and each stick is then out into three sections without being displaced from its position. The group is then sub-sampled by taking the first, fourth, seventh, *i.e.*, from the " top " sections, the second, fifth, eighth, etc., from the ' middle ' sections and the third, sixth ninth, etc., from the " root " section. These bundles are kept separate. Top and bottom halves of alternate sections are fibrated. (ii) Sticks are laid side by side half with butts facing alternatively in opposite directions, and each stick is marked with Chalk or Knife cut as nearly as can be judged at the middle point. A top half and a butt half are fibrated alternatively stopping each time at the marked centre and discarding the half which has served as a handle. Each stick originally selected should be a whole cane. If any stick be inconveniently long, part of the half not to be fibrated may be removed, leaving a foot or so to serve as a handle, which should be discarded after fibrating of the eligible half. The fibrated portion of each stick should be as nearly as possible a true half.

The fibrated material is thoroughly and quickly mixed, and a portion weighed out for analysis. Every precaution must be taken to keep the edges of the fibre top in a sharp condition and to maintain the necessary

speed to ensure that the cane treated will be finely and satisfactorily shredded.

One hundred grammes are weighed into calico bags placed in suitable tins to prevent evaporation while weighing. The bags are placed in cold running water and squeezed by hand several times during one hour, and then they are immersed in circulating boiling water for one hour, during which time they are removed and squeezed four times. The bags are then pressed to eliminate surplus water and dried to a constant weight at a temperature of from 100 to 105 degrees Centigrade.

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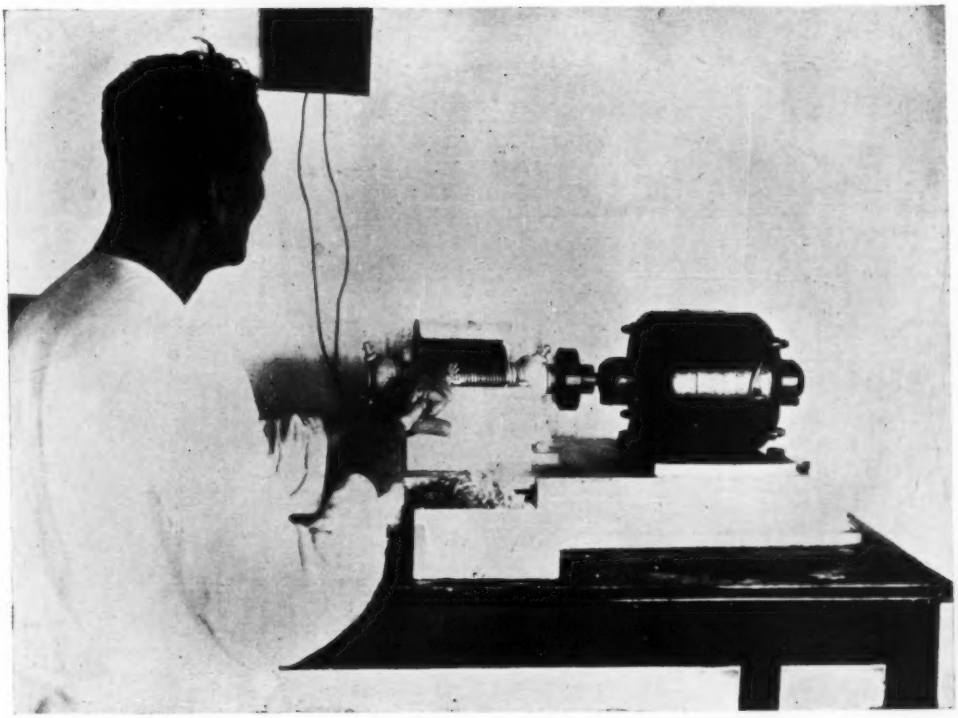

Showing Electric Shredder employed in Fibre Estimation work

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